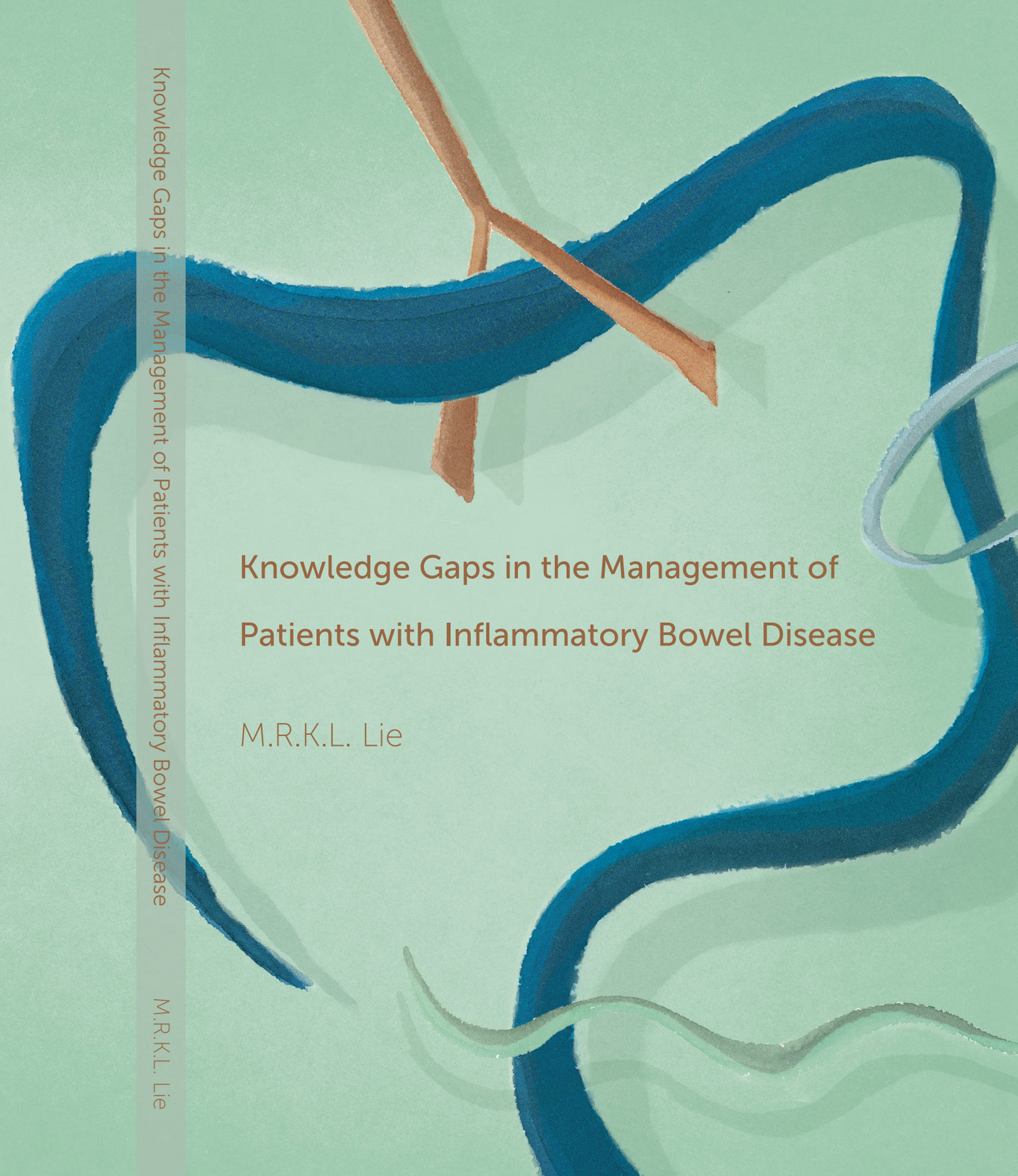
Knowledge Gaps in the Management of Patients with Inflammatory Bowel Disease

M.R.K.L. Lie



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Kennishiaten binnen behandeling van patiënten met een inflammatoire darmziekte

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General introduction

Inflammatory bowel disease

Inflammatory bowel diseases (IBD) are a heterogeneous group of diseases consisting of chronic, often relapsing-remitting, intestinal inflammation. IBD is subdivided in two overarching disease types with different patterns of inflammation, namely Crohn's disease (CD) and ulcerative colitis (UC). Both diseases can be subdivided based on the extent of intestinal inflammation using classification systems such as the Montreal classification[1].

The exact pathogenesis of IBD remains elusive, but the current paradigm consist of a complex interplay of genetic susceptibility[2-3], environmental factors[4-5] and dysfunction in the interaction between gut microbiome and the immune system[6].

In the last decades, the incidence of IBD has increased in the Netherlands, which is also seen worldwide[7-8]. With the Dutch rise in incidence, the prevalence of IBD has also increased, currently resulting in approximately 80.000 – 100.000 patients in the Netherlands.

As a chronic disease, the chronic treatment for IBD results in significant healthcare costs for society[9], particularly since IBD is often diagnosed at a relatively young age, with the incidence peaking at the ages of 15-30 years old[10]. Furthermore, IBD can negatively affect the quality of life of patients, due to factors such as chronic fatigue[11], hospitalizations and surgery[12], sexual dysfunction[13] or negative body image. Any of these factors may also reduce work productivity[14-15], resulting in an increased burden of IBD on society as a whole.

Despite advances in diagnostics and discoveries of new therapies, no definitive cure currently exists for IBD. As such, the current aim of treatment is reduce patient symptoms via suppression of gut inflammation, also known as achieving quiescent disease or mucosal healing. Suppression of gut inflammation not only leads to fewer symptoms, but also reduces the risks of complications such as strictures, malnutrition and colorectal carcinoma[16]. In recent years, the therapeutic arsenal to achieve mucosal healing has expanded and continues to expand due to continued new insights into the pathogenesis of IBD and the underlying immune pathways involved once the diagnosis has been established. Nevertheless, only approximately 60% of IBD patients manage to achieve long lasting remission with the drugs currently at our disposal[17].

The current treatment paradigm in IBD consists of a treatment pyramid, with several tiers. The different tiers are based on both the efficacy and toxicity of a drug. Drugs with with relatively few side-effects and low toxicity are present in the lowest tiers, whereas the highest tiers consist of powerful or invasive treaments with potentially severe side effects. Most patients are treated succesfully with drugs from the first tier, but in case of treatment failure a 'step-up' to the next tier is performed until gut inflammation is completely suppressed. However, an individualized approach of each patient is key, as there is considerable heterogeneity amongst patients, due to differences in disease behaviour and response to therapy.

Aims and outline of this thesis

Despite ongoing research, many knowledge gaps in the pathogenesis, etiology and treatment of IBD remain. The aim of this thesis is to fill some of these knowledge gaps, as outlined below.

In Chapter 1 we provide an overview of the current treatment guidelines for ulcerative colitis, with the aim of identifying patient groups not covered by guidelines.
For instance, a subset of UC patients exists who have inflammation limited to the rectum, which is referred to as ulcerative proctitis according to the Montreal classification. Due to the limited extent of inflammation in these patients, the current guidelines make specific treatment recommendations, with the first line treatment being topical 5-ASA compounds (i.e. locally applied via suppository or enema)[18]. In case of failure or intolerance, the guidelines further recommend prescribing topical corticosteroids, though the evidence for this recommendation is limited. However a good synthesis of the efficacy of these therapies is lacking. Therefore in Chapter 2 we systematically assess the evidence for the use of topical 5-ASA and corticosteroids in ulcerative proctitis, using a systematic literature review and meta-analysis.

However, even when the recommended topical treatments are employed, inflammation may persist, with some studies reporting up to 55% of patients with refractory inflammation[19]. For these patients, other therapies are necessary to induce disease remission. However, how to manage these refractory proctitis patients remains a significant knowledge gap. Though new drugs for ulcerative colitis in general have been studied and approved in recent years, patients with only proctitis are actively excluded from these corresponding 'landmark' trials[20-21]. Thus, the efficacy of new

drugs in patients with proctitis remains unclear. Furthermore, these drugs are administered systemically, whilst the inflammation is limited to less than 20cm of the colon. As such, another local therapy would be highly preferable to prevent systemic drug exposure.

Several small studies have shown that topically applied tacrolimus, a known effective immunomodulatory drug, is an effective treatment for ulcerative proctitis[22-23], however these studies have not compared tacrolimus with an active drug. In Chapter 3, we describe the results of a randomized clinical trial comparing the efficacy of topical tacrolimus with topical corticosteroids, in 5-ASA refractory proctitis patients.

In recent years, the increase in our understanding of the immune pathways involved in IBD has led to the identification of new therapeutic targets. However, IBD remains an incurable disease. As such, there is ongoing development of novel drugs interacting with these various inflammatory pathways[24-25]. However, not all IBD treatments involve newly discovered drugs as occasionally immunomodulatory effects are found in drugs that already exist. This strategy of 'drug repurposing' has been successfully employed in a variety of fields including cancer and urology[26-27] and remains of interest to all medical fields because the costs and time involved in developing a completely new compound can be prohibitive[28-29].

In this fashion, the drug naltrexone was shown to have immunomodulatory effects. This drug was originally designed to treat opioid withdrawal symptoms, via manipulation of the μ-opioid receptor[30], but recent studies have shown that lower doses of naltrexone lead to anti-inflammatory effects via modulation of endoplasmic reticulum stress[31]. Small studies showed a possible effect in animal studies[32] therefore in Chapter 4 we describe our investigations into the in vitro and in vivo effects of naltrexone in IBD.

Though many symptoms of IBD are directly related to gut inflammation, for instance diarrhea or bloody stools, patients may also experience other symptoms such as arthropathy or eye disease. In fact, such extra-intestinal manifestations occur frequently, with studies reporting a wide range of prevalences from 5% to 40%[33-34]. Due to the frequent occurrence of extra-intestinal complaints, the current guidelines supply recommendations for many of these symptoms[35]. However, an often overlooked symptom in IBD patients is severe fatigue. Severe fatigue is more frequently seen in patients with active intestinal inflammation, but even in patients with quiescent disease severe fatigue can persists in up to 40%[36-38]. Adequate treatment of fatigue remains relevant due to the relationship between fatigue and quality of life and general

productiveness[9,39-40]. In Chapter 5, we summarize the available knowledge on fatigue in IBD and develop a management algorithm designed to optimally guide and treat patients with debilitating fatigue.

As described in the guidelines, the goal of therapy in IBD patients is complete suppression of inflammation and not merely symptom control, as symptom control correlates poorly with actual control of inflammation. Since active inflammation is strongly associated with various long-term complications, the goal of therapy remains complete and maintained suppression of gut inflammation[41]. This goal is most often achieved using drug therapy, in some cases with just one drug, in other cases with a combination of drugs. However, though all currently employed IBD drug therapies are effective at controlling gut inflammation, all drug therapies are equally associated with toxicity[42-44]. Therefore the optimal drug dose is a balancing act between achieving optimal efficacy with as low as possible toxicity.

The process of determining the optimal drug dose usually starts in pre-clinical studies involving healthy volunteers, with subsequent modelling of the drug's pharmacokinetic properties. Often, these pharmacokinetic analyses are repeated in patients to confirm or optimize the models. In this fashion, the pharmacokinetics of several drugs prescribed for IBD have been studies[45-46]. However, the pharmacokinetics of the drug adalimumab have been examined less extensively. As such, in Chapter 6 we describe our investigations into the pharmacokinetics of adalimumab in a cohort of Crohn's disease patients.

Other than pharmacokinetics, there are many factors that could influence the disease course in IBD patients and the efficacy and toxicity of drug therapies. For instance, smoking[47], dietary factors[5] and lifestyle factors[48] are associated with the pathogenesis and/or the long term behaviour and severity of the disease. However of all patient factors, patient sex is infrequently analysed, even though there are signs that it plays a role in the pathogenesis and disease course of IBD[49-51]. Of note, patient sex refers to the biological sex (i.e. the presence of male or female reproductive organs), and should not be confused with the social and culturally constructed gender. We present our analysis of the influence of patient sex on the outcomes of adalimumab therapy in a cohort of Crohn's disease patients in Chapter 7.

In Chapter 8 we investigate the possible role of patient sex on drug efficacy further, using a systematic literature review and meta-analysis. In this study we specifically examine the efficacy of biological therapies, as measured as objectively as possible via

endoscopic disease assessments. Furthermore we investigate if patient sex is related to the occurrence and severity of drug related adverse events.

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Chapter 1

Managing ulcerative colitis: the guidelines and beyond

MRKL Lie, CJ van der Woude

European Medical Journal, Gastroenterology, 2013

Abstract

Management guidelines offer clinicians clear, evidence based and often succinct treatment advice. For ulcerative colitis these guidelines describe the use of 5-ASA, corticosteroids, thiopurines, cyclosporine and anti-TNF-alpha therapies. However, guidelines do have some drawbacks, mainly a lack of concrete advice concerning patients resistant to these aforementioned therapies. This review gives a short overview of current guidelines and addresses treatment alternatives for conventional therapies.

Introduction

The management of ulcerative colitis remains challenging to even the most seasoned clinician. This is partly due to the nonelucidated etiology of the disease. Periodically updated guidelines are valuable instruments that aid clinicians in decision making. However, the management of ulcerative colitis at an individual level remains challenging due to highly variable disease presentations that are not specifically covered by the guidelines. Specifically, patients intolerant to conventional therapy or with treatment resistant disease limited to only the rectum can make decision making difficult. Also, a patient's preferences for certain treatments can result in more complicated decision making, for example when patients refuse certain drugs or surgery.

In this review we will summarize the latest guideline on the management of ulcerative colitis. Next, the treatment options and evidence for patients that have exhausted the therapies suggested by the guidelines will be discussed and a strategy will be proposed for this particular subgroup. Furthermore, the limited evidence of several new biological therapies close to registration and approval will be examined.

Therapies for acute remission induction

The choice of therapy depends on disease severity and localization. To properly describe severity and localization, several classification systems exist. Most often the Mayo score or the Truelove and Witts' index is used to classify severity, whereas localization is usually anatomically described as proctitis (rectum only), left-sided (beyond the rectum but distal of the splenic flexure) or extensive (extending beyond splenic flexure). Below are summarized the appropriate conventional treatments. The 2012 European Crohn's and Colitis Organisation (ECCO) guidelines on ulcerative colitis give more thorough recommendations in different situations[1].

Proctitis

Topical 5-ASA therapy is the first line therapy for proctitis. There is good quality evidence for topical treatment only[2-12], with some evidence showing that topical 5-ASA treatment is superior to oral 5-ASA treatment alone[13]. Topical steroid therapy has been found to be inferior for remission induction[14] and should therefore be used as a second line therapy in case of 5-ASA intolerance.

Left sided disease

A combination of oral and topical 5-ASA has proven to be more effective than either agent alone in the treatment of left sided ulcerative colitis.[15-18]. If this fails oral steroids might be added.

Extensive disease

Combined oral plus topical 5-ASA remains the first line of treatment. If this therapy fails, oral steroids can be added[19-23]. If steroid dependence occurs, thiopurine treatment is recommended[24].

Severe disease

Severe disease is potentially life-threatening and in most cases requires hospital admission and immediate treatment. All guidelines recommend high dose intravenous glucocorticoids as the first treatment modality, even though only limited evidence exist[25-27]. Of great importance is early consideration of salvage treatments as a precautionary measure as the patient may not respond to steroid treatment.

Intravenous steroid-refractory severe disease

Intravenous steroid-refractory disease leaves clinicians with limited drug therapies. Salvage therapy should not be initiated simply to delay surgery, as such delays will lead to greater morbidity at surgery[28]. If clinical and biochemical parameters allow an attempt at salvage, the guideline recommends cyclosporine, infliximab or tacrolimus. High quality prospective evidence exists for the use of cyclosporine[27,29-31], confirmed by several retrospective studies[32-34]. There is also prospective evidence[31,35-37] and some retrospective evidence[34] for infliximab as a rescue therapy. The prospective evidence for tacrolimus is less extensive[38-40], containing heterogeneous populations and the use of tacrolimus is therefore not as strongly recommended by the guideline.

There is limited evidence for using infliximab as a rescue therapy to cyclosporine, or vice-versa[41,42]. The guideline recommends such a third line therapy only in select cases treated by a multi-disciplinary team in specialist centers.

Treatments and alternatives for steroid dependent disease

Though intravenous steroid-refractory disease represents the most severe cases of UC, this presentation is relatively rare. In contrast, it is more common to see outpatients who reach remission but either fail to taper their steroids or relapse soon after

tapering, making them steroid dependent. In the following paragraph several options for the treatment of steroid dependent disease and their respective evidence will be discussed.

Thiopurines

A prospective study[43] has shown that azathioprine (AZA) and its metabolite 6-mercaptopurine (6-MP) are highly effective in achieving steroid-free remission, with persistent long-term results found in observational studies[44].

Anti-TNF-alpha

In case of failure or intolerance to thiopurines, anti-TNF-alpha therapy is considered the next step. Several large trials[45] and a Cochrane meta-analysis[46] have conclusively proven the efficacy of infliximab in this setting. Though less extensively studied[47,48], adalimumab has also show efficacy in steroid dependent disease and in patients intolerant to thiopurine treatment.

Unconventional therapies (see Figure 1.1 for proposed algorithm)

If conventional therapies fail, colectomy becomes a valid treatment option for patients with UC. Clinical experience shows a profound difference in acceptability of colectomy in hospitalized patients compared with outpatients, though no formal studies have examined this issue. It is not uncommon for outpatients to refuse colectomy, despite being informed of the possible benefits of such intervention. In these situations a clinician may need to resort to either enrollment in clinical trials or initiation of an unconventional therapy in the hope of controlling a patient's symptoms. The provided algorithm (Figure 1.1) may help clinicians in their decision making regarding these therapies, which are described in more detail below.

Therapy resistant proctitis

A subset of patients with disease limited to the rectum is surprisingly treatment resistant to topical 5-ASA and / or topical steroid therapies. This may present clinicians with a treatment dilemma: escalate to systemic therapies, with all associated adverse effects, or accept the limited disease localization. There is a paucity of prospective controlled trials within this patient subgroup.

There is only one randomized, placebo controlled trial remotely addressing this issue[49]. This study investigated the efficacy of cyclosporine enemas in left-sided disease

(disease extent ranging from 10 to 60cm ab ano). No significant difference in remission rate between cyclosporine and placebo was found.

Two open label pilot studies investigated the efficacy of topical tacrolimus for treatment resistant proctitis. The first[50] applied tacrolimus ointment in ulcerative proctitis patients who failed previous 5-ASA, steroid, immunosuppressant and infliximab therapy. 75% (6 out of 8) achieved remission after 8 weeks, with reduction or cessation of steroid usage in 5 of the responders. The second[51] treated 12 patients with ulcerative proctitis resistant to topical 5-ASA and/or topical steroid therapy. This study used tacrolimus suppositories and assessed efficacy after 4 weeks of treatment. Clinical remission was achieved in 83% (10 out of 12) with complete endoscopic healing in 33% (4 out of 12). These promising pilots warrant further investigation of topical tacrolimus in treatment resistant ulcerative proctitis.

Even retrospective data is scarce. One study[52] retrospectively investigated the efficacy of infliximab in patients with proctitis resistant to at least 5-ASA and steroids. Clinical response was seen in 85% (11 out of 13) after infliximab induction therapy. Two patients suffered from adverse events. Other retrospective studies[53-55] regarding infliximab contain only a few subjects with proctitis, and their response is not individually reported.

Mycophenolate mofetil

No randomized studies have been performed, but the results of 1 retrospective and 3 open-label prospective studies have been published. The first study[56] retrospectively examined the effectiveness of MMF in 70 steroid dependent IBD patients, of which 19 had UC. After an unclear treatment time (the average treatment time amongst all study subjects was 28 months), 35% (6 out of 17) of UC patients was in steroid-free remission. 65% (11 out of 17) failed to respond to MMF or were intolerant.

The 3 prospective studies consist of two uncontrolled, open-label studies and one unblinded pilot study. The first open label study[57] examined 24 IBD patients, of which 13 had UC with moderate to severe steroid dependent disease. Patients were treated with combined MMF and high-dose steroids with tapering. In the first 3 months, 46% (6 out of 13) patients achieved remission, but after steroids were tapered, the disease relapsed in all UC patients. The other open label study[58] treated 14 patients with IBD resistant to conventional therapy. They included 5 patients with UC (or IBD unclassified), all of which were steroid dependent and intolerant to thiopurines. One

patient suffered from side effects and ceased MMF treatment, the other 4 reached remission at 8 weeks and ceased steroid treatment. Follow-up at 12 months showed a maintained remission in 67% of all patients, but the exact data for UC patients at that time point is not reported.

Lastly, in the only controlled study[59] MMF was compared to azathioprine in 24 UC patients. Both groups received steroids in a tapering dose. Notably, this study excluded patients with current steroid usage. After 4 weeks of treatment, 67% (8 out of 12) in the MMF group reached remission and 5 remained in remission throughout the whole follow-up period of 1 year. However during the entire study, the remission rates were higher in the azathioprine group than in the MMF group, though no significance value is provided by the authors.

Methotrexate

Little prospective studies have been performed on methotrexate (MTX) in ulcerative colitis. One study in 1996[60] examines the effectiveness of MTX versus placebo in steroid dependent ulcerative colitis. No difference in remission rates was found (47% in the MTX group), which is similar to the results of several case series[61-63] (45 to 54%). However it has been argued[64-65] that the studied dose of 12.5mg / week is considerably lower than the “modern” dose of 20 to 25mg / week.

Upcoming results of the French METEOR study and the North American MERIT-UC study may shed some light on the use of MTX in UC. Both investigate the effectiveness of MTX 25mg / week for remission induction in treatment resistant and/or steroid dependent ulcerative colitis. It should be noted that whilst according to www.clinicaltrials.gov the MERIT-UC study is currently recruiting, the METEOR study already ended in November 2010, but as of yet no results have been published.

Tacrolimus

Several retrospective studies[66-73] have analyzed the effects of tacrolimus on severe, therapy resistant ulcerative colitis. Outcome parameters, concomitant medication, tacrolimus dosage and target trough levels varied amongst these studies. However all studies show a high clinical response rate, varying between 61% to 90%. Reported clinical remission rates vary between 33% and 72%.

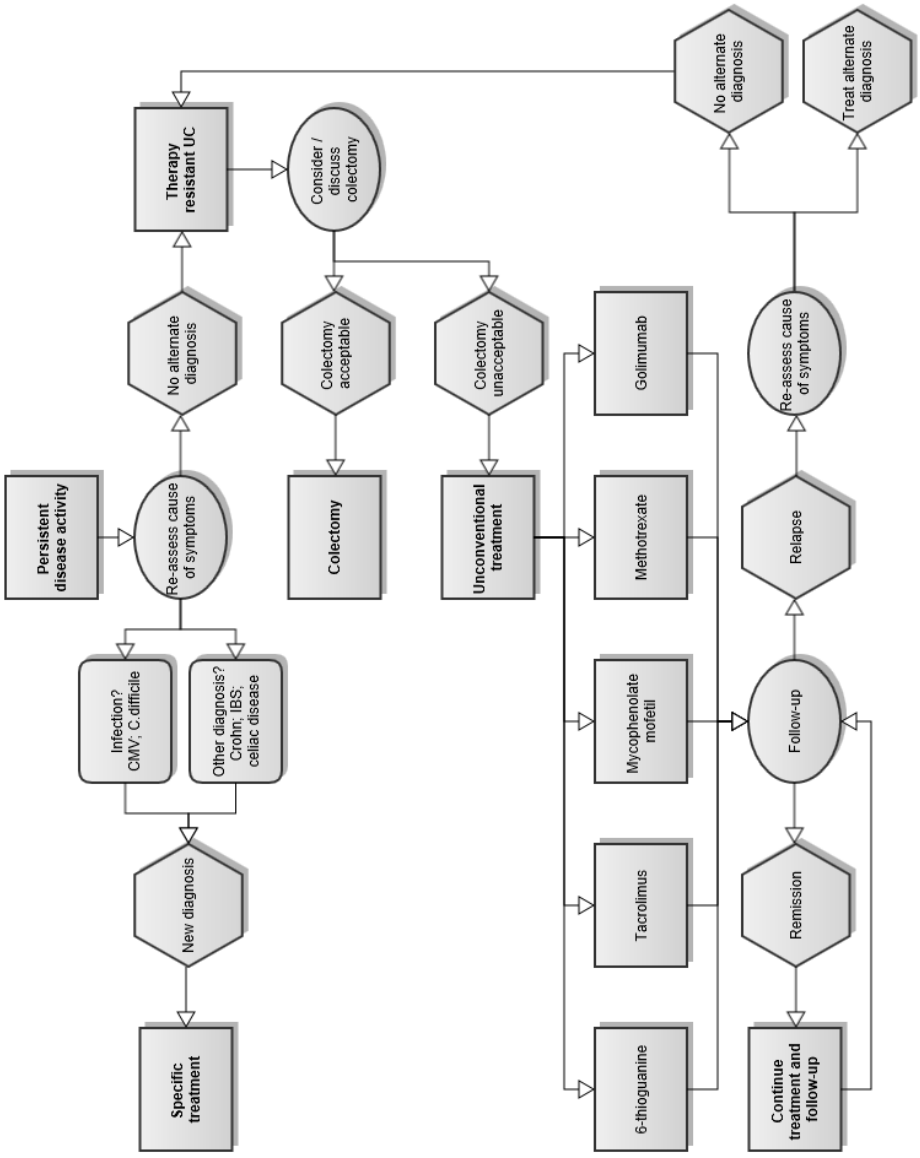
Persistent disease activity
Re-assess cause of symptoms
Infection? CMV; C. difficile
Other diagnosis? Crohn; IBS; celiac disease
New diagnosis
Specific treatment
No alternate diagnosis
Therapy resistant UC
Consider / discuss colectomy
Colectomy acceptable
Colectomy
Colectomy unacceptable
Unconventional treatment
Golimumab
Methotrexate
Mycophenolate mofetil
Tacrolimus
6-thioguanine
No alternate diagnosis
Treat alternate diagnosis
Re-assess cause of symptoms
Relapse
Follow-up
Remission
Continue treatment and follow-up

Figure 1.1 Management algorithm for therapy resistant ulcerative colitis. UC ulcerative colitis; CMV cytomegalovirus; IBS irritable bowel syndrome.

The only randomized controlled trial[38] concerning tacrolimus in UC randomized 62 patients with steroid refractory, moderate to severe UC. Changes in the tacrolimus dose were made to achieve a target trough level of 10-15ng/mL. This study shows a 50% clinical response at two weeks, with a clinical remission rate of 9% (3 out of 32), with greater response amongst patients who reached the target trough level. After a two week open-label extension period, the clinical remission rate increased to 29% (6 out of 21).

6-Thioguanine

6-Thioguanine (6-TG) is a metabolite of 6-mercaptopurine. Because of polymorphisms in the enzyme thiopurine methyltransferase, the conversion of 6-MP to 6-TG can differ markedly between patients. Directly administering 6-TG should therefor remove dosing issues whilst in theory achieving similar results to AZA and 6-MP treatment. However there is little published data that studies 6-TG treatment directly. Of additional interest is the use of 6-TG in patients with intolerance to AZA or 6-MP. An open label pilot study was performed in 49 patients with Crohn's disease, of whom 23 patients had pancreatitis after AZA or 6-MP administration[74]. None of these patients had recurrence of their pancreatitis after switching to 6-TG.

Table 1.1 Recommended dosage, laboratory tests and absolute contra-indications for 6-thioguanine, tacrolimus, mycophenolate mofetil and methotrexate.

Agent	Dosage	Contraindications	Laboratory and functional tests		Comments
			Preliminary	Follow-up	
6-thioguanine	OD, oral, 0,3 mg/kg	Liver insufficiency Pregnancy	CBC, LF, RF	CBC, LF	Consider TPMT enzyme activity testing Reduce dose in renal impairment Reduce dose if concomitant allopurinol
Tacrolimus	OD, oral, 0,1 mg/kg	Liver insufficiency	ECG, CBC, LF, RF	CBC, LF, RF, TL	Aim for trough level 4-8 ng/mL
Mycophenolate mofetil	BD, oral, 500-1000 mg	Pregnancy	CBC, LF, RF	CBC, LF, RF	Adjust dose based on CBC
Methotrexate	QWK, SC, 25 mg Reduce to QWK 15mg after 12 weeks	Renal impairment (GFR < 20 mL/min) Pregnancy	CBC, LF, RF	CBC, LF, RF	Also prescribe QWK 5 mg folic acid Adjust dose based on CBC
Abbreviations: OD once daily; BD twice daily, QWK once weekly, SC subcutaneous, GFR glomerular filtration rate, CBC complete blood count, LF liver function, RF renal function, TL trough level, TPMT thiopurine methyltransferase					

A database analysis[75] was performed regarding UC patients receiving 6-TG after becoming intolerant to conventional thiopurine treatment and/or being steroid dependent. 46 UC patients were examined, of which 83% (37 out of 46) were on steroids when 6-TG therapy was initiated. 80% (37 out of 46) of patients remained in remission after a median follow-up time of 22.4 months, 13% (6 out of 46) were

intolerant and the remaining 7% (3 out of 46) failed therapy and underwent colectomy. The amount of patients in steroid-free remission is not described.
A prospective, open-label study[76] treated 16 UC outpatients who had steroid dependent or refractory disease. After 3 months, 31% (5 out of 16) had complete response and 38% (6/16) a partial response.
The measurement of 6-TG levels in the setting of monitoring AZA and 6-MP therapy has been studied extensively and has been found to be useful in meta-analyses[77]. If these results are extrapolated to direct treatment with 6-TG, it is likely that the clinical efficacy of 6-TG is similar to AZA and 6-MP treatment, as long as sufficient serum levels are achieved.

Summary regarding disease resistant to conventional therapies

When treating patients with UC resistant to conventional therapies, the first step is to ensure that it is indeed the UC that is causing the symptoms. Critical re-assessment of the patient to rule out any other pathology is highly important. Secondly, good communication is key since the "rescue" therapies described above have low remission rates and only weak supporting evidence. Patients should be well informed on the potential benefits and risks of these agents. Specifically, patients should be aware that failure of these therapies will increase the likelihood of requiring colectomy.

Figure 1.1 summarizes our recommendations, whilst Table 1.1 shows recommended dosage, laboratory tests and contra-indications. 6-TG and tacrolimus have the highest reported remission rates, therefor we would recommend these agents over MMF, MTX or LDN. The other 3 agents are still useful in specific circumstances, for instance LDN is the most suitable agent for females who wish to become pregnant.

We strongly recommend that all the above drug treatments should be accompanied by close follow up in order to detect treatment failure in a timely fashion. Laboratory markers such as fecal calprotectin, reflecting intestinal inflammation[78,79], may aid in the follow-up process. In case of treatment failure or clinical deterioration re-assessment should ensue, after which optimizing therapy, switching therapy or, if necessary, colectomy should follow.

Future therapies

A search in the U.S. National Institutes of Health clinical trial database (http://clinicaltrials.gov) using the term "ulcerative colitis" yields 169 planned or active studies. 29 of these studies involve new compounds, which reflect the continuing

interest of many pharmaceutical companies regarding treatment for UC. These compounds are still only known by their study names and mostly involve phase 1 and phase 2 studies, with no results currently available on the website. Amongst these drug candidates are OKT-3 (an oral anti CD-3 agent), ASP3291 (a melanocortin receptor agonist), KRP203 (a sphingosine-1-phospate receptor modulator), GWP42003 (a cannabinoid), AMG181 (an α4β7 integrin antibody), HE3286 (a synthetic steroid derivative), GL1001 (an ACE-2 inhibitor) and MDX1100 (an CLCL10 antibody). It is anticipated that their role in UC will become clear in the near future.

Not all new and promising therapies live up to our expectations. For instance, basiliximab, daclizumab and visilizumab were promising in uncontrolled pilot studies[80-84], but eventually showed identical remission rates to placebo in randomized controlled trials[85-87].

Golimumab

Golimumab is a fully human antibody against TNF-alpha. At the DDW in 2012, the initial results of the PURSUIT-SC trial regarding golimumab in UC were presented. Recently the complete article on this two-part, randomized, double blind, placebo controlled phase 2-3 study has been published[88]. A total of 1064 patients were included, 291 in the phase 2 dose-ranging study, 774 in the phase 3, efficacy study. All patients had moderate to severe UC and an inadequate or failed response to at least 1 conventional therapy. The efficacy study evaluated clinical response after 6 weeks of treatment which was achieved in 53% (275 out of 515) of the golimumab groups versus 30% (76 out of 256) of the placebo group. Clinical remission at 6 weeks was 18% (94 out of 515) for the golimumab groups versus 6% (16 out of 256) for the placebo group.

At least one study is planned to examine the efficacy in pediatric patients, whilst another study in Japan is recruiting patients. These studies will address the reproducibility of the results found in the PURSUIT-SC study, though its results have already led to FDA approval for golimumab in moderate to severe UC in May 2013.

Vedolizumab

Vedolizumab is an antibody to the α4β7 integrin heterodimer complex. 3 studies have been published on its efficacy in ulcerative colitis. The first study[89] reported results of a randomized controlled trial performed in 181 patients. Patients were either untreated or had only received 5-ASA therapy. Vedolizumab or placebo was administered on day

1 and day 29. Clinical response rates were 66% and clinical remission was achieved in 33% at 6 weeks of follow-up.

Two other studies[90,91] on vedolizumab were a randomized controlled dose-ranging study, and an open label extension of the first, with additional enrollment of treatment naïve patients. In the controlled trial 47 patients with moderate, but not steroid resistant, UC participated and medication or placebo was administered on day 1, 15, 29 and 85. Clinical response at 16 weeks was 60% to 80% (depending on dose). Clinical remission is reported as varying from 53% to 79% between day 29 and 253, compared with 25% to 50% in the placebo group. The study was underpowered for assessment of clinical outcome. The open label extension study involved 72 patients with UC who were administered vedolizumab on day 1, 15, 43, followed by maintenance dose every 8 weeks. After 70 weeks of follow-up, clinical response was achieved in 92% and remission in 77% of patients with moderate to severe UC.

Recently the results of the GEMINI study, a multi-center, randomized, double-blind, placebo-controlled trial were published[92]. This study involved 2-phases, with 895 patients in the induction and maintenance phase combined. Notably, patients had active disease and had failed previous glucocorticoid, immunosuppressive or anti-TNF-alpha therapy , though disease limited to the rectum was an exclusion criterion. After 6 weeks, coinciding with the end of the induction phase, vedolizumab showed a statistically significant 47% clinical response rate compared with 26% for placebo. The maintenance phase ended after 52 weeks, again showing a significant difference in clinical remission rates with 42% and 45% for vedolizumab in different doses, compared with 16% for placebo.

No current trials on vedolizumab were identified, but a request for FDA approval was filed in June 2013, most likely based on the results of the abovementioned studies.

Tofacitinib

Tofacitinib is an oral inhibitor of Janus kinase 1, 2 and 3, and it's effect should result in reduction of interleukin 2, 4, 7, 9, 15 and 21. The results of a large, multicenter, randomized, double-blind, placebo-controlled trial were published in 2012[93], examining the efficacy of tofacitinib in patients with active UC. A total of 194 patients were randomized between 5 groups, 1 placebo group and 4 groups with different tofacitinib dosage (0.5mg, 3mg, 10mg and 15mg twice daily). 34% of patients were using

concomitant steroids, whilst 27% were steroid resistant and 19% had failed anti-TNF therapy.

Significant difference in clinical remission was seen in the 3mg, 10mg, and 15mg groups compared with placebo, with remission rates of 33%, 48%, 41% compared with 10% respectively. Endoscopic remission showed similar significant differences, with 18%, 30%, 27% compared with 2% in the placebo group.

Regarding clinical and endoscopic response, only the highest tofacitinib dose showed a significant difference compared with placebo. Clinical response was 78% compared with 42%, whilst endoscopic response was 78% versus 46%.

Currently, the OCTAVE study is recruiting UC patients to analyze the efficacy in moderately to severely acute UC, resistant to at least corticosteroids, azathioprine or anti-TNF therapy. It consists of a remission induction phase, examining efficacy at 8 weeks, and is followed by a long-term follow-up study of 52 weeks.

Discussion

In this paper we have reviewed the most recent guideline by the European Crohn's and Colitis Organization on the treatment of ulcerative colitis. The proper evidence based approach is described extensively in the guideline, and we underscore its usefulness in clinical practice. Nevertheless, it remains challenging for clinicians to extrapolate the results obtained in clinical trials to individual patients.

When patients become resistant to conventional therapies, the situation moves beyond the guidelines, and it is for these situations that we offer the treatment algorithm described above. Of utmost importance remains the individualized and tailored approach, based on the patient's preference, the clinician's preference and the availability of therapies. The choice of these unconventional therapies should be made in conjunction with the patient, underscoring the need for clear communication between clinician and patient, regarding the pros and cons of each treatment modality.

Finally, though the primary aim of these therapies are the induction and maintenance of remission, and subsequently the avoidance of surgery, one could also consider these agents as a bridge to novel treatments, either those substances currently awaiting regulatory approval or those in the last stage of their development.

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Chapter 2

Drug therapies for ulcerative proctitis – systematic review and meta-analysis

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Inflamm Bowel Dis. 2014;20(11):2157-78

Abstract

Background

Patients with ulcerative colitis limited to the proctum are considered to have ulcerative proctitis (UP). In patients with more extensive ulcerative colitis, treatment occurs in a step-up fashion (5-ASA, corticosteroids, thiopurines, anti-TNF-a agents), a strategy which has proven effective. Although treatment of UP occurs using the same step-up design, the efficacy of these therapies in UP is scarcely studied. The objectives were to systematically review the literature for randomized controlled trials studying drug therapies for induction and maintenance of remission in patients with UP.

Methods

Electronic databases and reference lists of review articles were searched. The primary outcomes were clinical remission induction rate and the maintained clinical remission rate. Secondary outcomes were induction and maintenance of endoscopic and histological remission. Relative risks (RR) and 95% confidence intervals (CI) for were calculated.

Results

Twenty-three studies (1834 patients) were included. Eighteen trials investigated induction and 5 studied maintenance of remission. Topical 5-ASA was significantly superior to placebo for induction (RR, 2.39; 95% CI, 1.63-3.51) and maintenance (RR, 2.80; 95% CI, 1.21-6.45) of clinical remission, regardless of dose or formulation. Subgroup analysis of 5-ASA suppositories also showed superiority over placebo for induction of clinical (RR, 3.07; 95% CI, 1.70-5.55) and endoscopic remission (RR, 2.64; 95% CI, 1.85-3.77).

Conclusions

Topical 5-ASA is superior to placebo for the induction and maintenance of clinical remission and for the induction of endoscopic remission. The efficacy of corticosteroids, thiopurines, and anti-TNFa has been insufficiently studied in patients with UP.

Background

Description of the condition

The incidence rate for ulcerative colitis (UC), a chronic inflammatory bowel disease varies from 0.5 to 24.5 per 100,000 person-years worldwide[1]. Ulcerative proctitis (UP) is defined as ulcerative colitis, with inflammation limited to within 12cm of the anal verge. Similar etiological factors are likely to precipitate distal and extensive colitis. Newly diagnosed UC cases in adults often present with disease limited to the distal or descending colon (left-sided ulcerative colitis, LUC), and the subset of patients with UP may encompass up to one-third of all UC cases[2,3].The course of UP varies and most patients experience symptoms in remission - relapse cycles.

In general, UC is classified based on the extent of the disease. Inflammation extending proximally beyond the splenic flexure is termed "extensive disease". This term includes "pancolitis" or "total colitis", both of which refer to inflammation of the whole colon. Inflammation up to the splenic flexure is classified as "left-sided disease" and less often as "distal disease".

The terms "extensive disease" and "left-sided disease" are generally well accepted and unambiguous. The terms used for more distal disease may vary amongst different manuscripts. Some report the extent of inflammation based on the anatomy of the distal colon, for example “disease limited to the rectum”, “up to the sigmoid” or “up to the ascending colon”. Other studies classify the extent as “proctitis” (5-25cm[4-5]), “procto-sigmoiditis" (15-40cm[6-7]) and "distal colitis" (5-60cm or up to the splenic flexure[8-9]).

During the initial assessment of patients with UC, as shown in Figure 2.1, the prevalence of UP may be as high as 44-60%[10]. Long-term epidemiological studies have revealed that UP often extends to more proximal colitis and even to total colitis. It was shown that nearly 22% of patients with UP had disease progression within 12-24 months of initial diagnosis despite medical treatment[11]. This is in line with other studies, showing proximal extension of UP in 28% during 5 years of follow up[2,12-13]. The recognition of UP and LUC is important because it has been suggested that effective local treatment may prevent or delay proximal spread of the inflammation[14].

Description of the intervention

Treatment of UP occurs in a step-up design, as shown in Figure 2.1. The first step of treatment is topical application of 5-aminosalicylic acid (5-ASA), administered as suppositories. It has been shown that 5-ASA induces remission in active proctitis and distal colitis in 31-80% of subjects (median 67%) compared to 7-11% of those given placebo, in a meta-analysis of 11 trials in 778 patients[15]. Despite the significant benefits of rectally administered 5-ASA, some patients will fail to improve on and will require additional medical therapy. The next treatment steps are a combination of oral and topical 5-ASA, adding corticosteroids (topical or systemic), followed by treatment with thiopurines and finally treatment with tumor necrosis factor-alpha (TNF-alpha) antibodies.

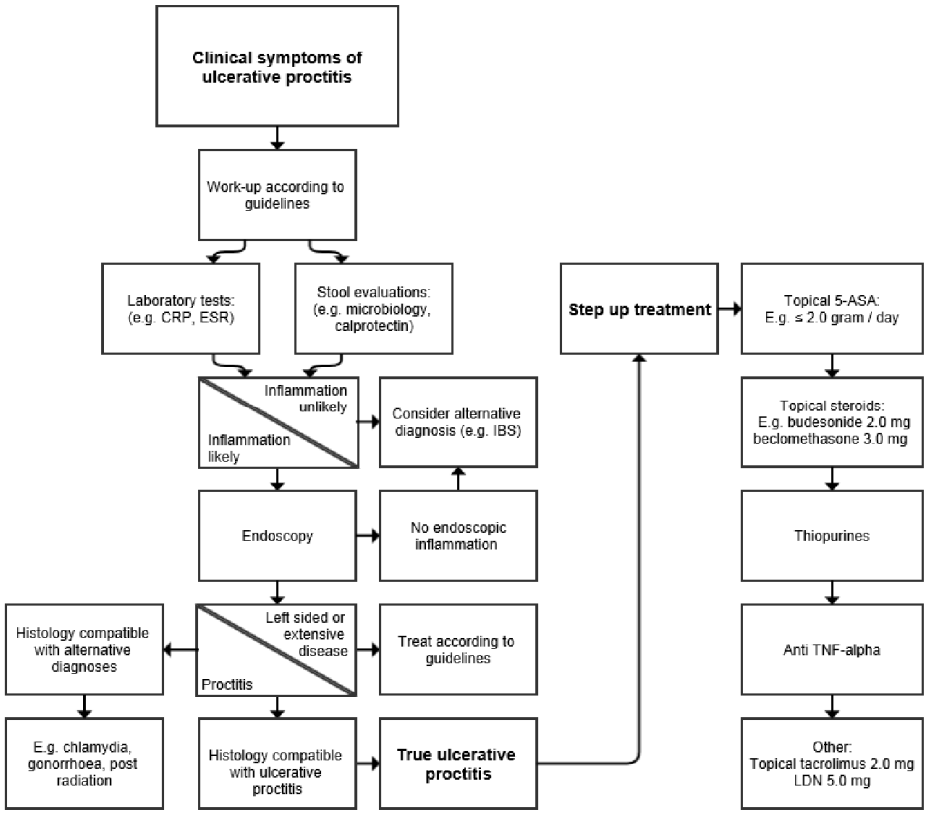

Figure 2.1 Diagnosis and treatment scheme for ulcerative proctitis.

How the intervention might work

5-ASA

The exact working mechanism of 5-ASA (also known as mesalazine and mesalamine) is unknown. Several in vitro studies have shown inhibition of cytokine synthesis, improved free radical scavenging and direct immunosuppressive effects of 5-ASA[16-18]. Of note, older studies often examine sulphasalazine (SASP) and olsalazine. The former is split by the colonic bacterial flora into the inactive compound sulphapyridine and the active substance 5-ASA. The latter consists of two 5-ASA molecules, whose chemical bond is broken down by colonic bacterial flora.

Corticosteroids

Corticosteroid formulations, both in oral and topical formulation, exert various effects on both the innate and acquired immune system. These effects include an increase in circulating neutrophils, a reduction in circulating B and T-lymphocytes and a reduction of cytokine synthesis by lymphocytes[19-22].

Thiopurines

The thiopurines azathioprine and 6-mercaptopurine are considered antimetabolites. They act as purine analogues that disrupt the nucleic acid metabolism, resulting in reduced cell proliferation[23]. Additionally, thiopurines have a direct inhibitory effect on T and B lymphocyte proliferation[24].

Anti-tumor necrosis factor alpha antibodies

TNF-alpha is a pro-inflammatory cytokine, produced by many lymphoid cells. It's effects include up regulation of the pro-inflammatory cytokines IL-1 and IL-6 and increased leucocyte migration[25]. Additionally, TNF-alpha itself is up regulated in various inflammatory diseases, including IBD. Inhibition of TNF-alpha will results in a decreased inflammatory response.

Why it is important to perform this review

Current treatment of UP occurs in a step-up design, similar to the treatment of UC. There is good evidence that supports this step-up design in UC. Meta-analyses have shown the efficacy of oral and topical 5-ASA in UC, both for induction[15,26-27] and maintenance of remission[28]. Meta analyses have also shown the value of

corticosteroids for remission induction[29], thiopurines for maintenance of remission[30] and anti-TNF for induction and maintenance of remission[31] in UC.

However, the evidence for these treatments in UP is not as clear. No meta-analyses of drug therapies in UP patients have been performed and the previously mentioned meta-analyses only assessed the efficacy of drug therapy in more extensive disease. Only the meta-analysis regarding topical 5-ASA had planned a subgroup analysis for UP patients, but it could not be performed due to the inability to extract sufficient subject-level information.

Objectives

To systematically review the literature for studies examining drug therapies for patients with ulcerative proctitis, both for remission induction and maintenance of remission and, where possible, determine the efficacy of these therapies trough meta-analysis.

The primary outcomes were induction and maintenance of remission.

Methods

Search strategy

A systematic database search for randomized controlled trials was performed on 28 April 2014, without restrictions on language, publication year or publication status. This search was performed in the following databases: Embase (including Medline), Medline OvidSP, Cochrane Central Register of Controlled Trials, Web of Science and Pubmed. The detailed digital search strategy is provided in digitally available appendix. Additionally, the reference lists of all potentially relevant articles were studied for further trials. Any studies found trough this search also had their reference lists studied.

Review and study selection process

Titles and abstracts identified through the search strategy were assessed by two independent reviewers (ML and SK) for potential eligibility, using pre-defined criteria as described in the appendix. Disagreements were settled in consensus and, if necessary, after discussion with a third independent reviewer (CW). The manuscripts deemed potentially eligible for inclusion were obtained for full text review. The full texts were

assessed by the two independent reviewers, using pre-defined eligibility criteria as described in the appendi. Discussions with the third independent reviewer were used to resolve disagreements.

Data extraction

Data from the eligible studies was extracted using a standardized form by the two primary reviewers. Differences in the extracted data were resolved through consensus or, if necessary, discussion with the third independent reviewer. For each study, the following data was extracted:

1. Study methods (including method of allocation and concealment, blinding, duration of study)
2. Participants (including age, extent of disease, duration of disease prior to enrollment)
3. Interventions (including dosage, duration, formulation)
4. Outcomes (including definition of clinical remission, remission and response rates).

Assessment of risk of bias in included studies

The risk of bias was assessed independently by each of the two primary reviewers, according to the scheme described in the Cochrane Handbook for Systematic Review of Interventions[32]. This assessment involved judgment on selection, performance, attrition and detection bias. The 'Risk of bias tool' in the publically available program RevMan 5.2 was used to report possible bias in included studies.

Data synthesis and statistical analysis

As the primary outcomes of all included studies were dichotomous, we used statistical methods as described by Yusuf et al.[33]. The results were expressed as risk ratio's with 95% confidence interval for achieving the outcomes specified above.

Where applicable, studies were pooled using a random-effects, regardless of statistical heterogeneity. Heterogeneity was tested using the Chi-squared test, the I-squared test and visual inspection of forest plots. If heterogeneity was present, we attempted to investigate the cause thereof (such as methodological factors or the outcome assessment). Given the limited number of included studies, subgroup analysis or meta-regression was not considered useful. In the case of high heterogeneity (I2>75%), studies were pooled only if the direction of their results was consistent.

Results

Results of the search

The literature search performed on 28 April 2014 identified 2412 citations, of which 1853 remained after removing double entries. After reviewing title and abstracts 1781 manuscripts were considered irrelevant (e.g. did not study UC, no drug therapy, case reports, abstract format only). This resulted in 72 potentially relevant studies. Examining the reference lists eventually resulted in the addition of 86 potentially useful manuscripts. In total, 156 manuscripts were assessed completely for eligibility (see Figure 2.2: flowchart), of which 133 studies were excluded for various reasons (see the appendix). The remaining 23 studies were included in this review (see Table 2.1: Characteristics of included studies).

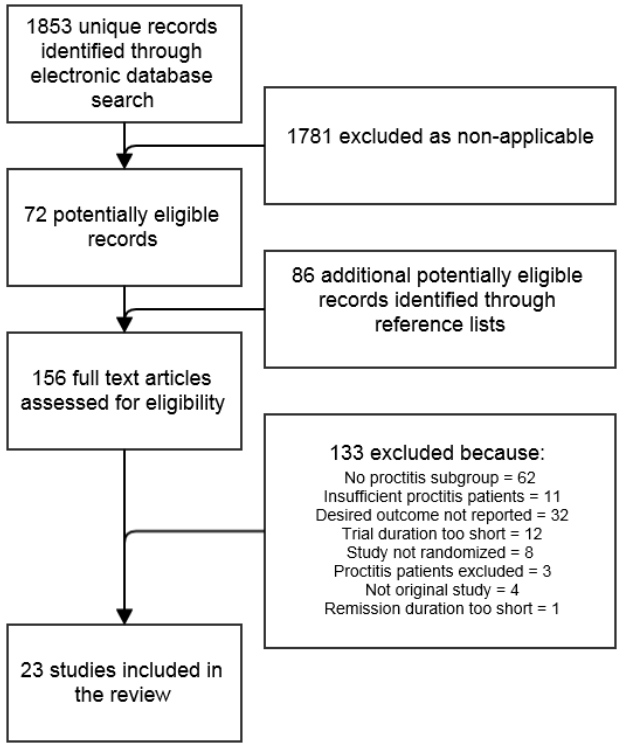

Figure 2.2 Flowchart of identification and selection of studies.

Table 2.1 Characteristics of included studies.

Andus 2010		
Methods	Randomized controlled trial, 2 parallel groups, investigator-blind	
Participants	403 patients, mean age 42 years, mean DAI at baseline 6.2 (for both groups) Diagnosis based on: endoscopy, histology and negative stool cultures	
Interventions	Both arms were treated for 6 weeks Arm 1: OD 1.000mg 5-ASA suppository (N=200) Arm 2:TID 500mg 5-ASA suppositories (N=203)	
Outcomes	Clinical improvement (at least 1 point decline in DAI) Clinical remission (DAI < 4 at final visit) Mucosal healing (mucosal DAI subscore 0 or 1) Endoscopic remission (EI < 4 at final visit) Histological remission (remission according to pathologist assessment)	
Notes	-	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Method of sequence generation not given
Selection bias (allocation concealment)	Unclear risk	Method of allocation unclear
Performance bias	Unclear risk	Participants were not blinded
Detection bias	Low risk	Outcomes assessed by blinded investigators
Attrition bias	Unclear risk	Used last observation carried forward, the actual amount of imputed data is not reported
Reporting bias	Low risk	-
Other bias	Low risk	-

Ardizzone 1996		
Methods	Randomized controlled trial, 2 parallel groups, investigator-blind	
Participants	40 patients, mean age 36 years Diagnosis based on: endoscopy, histology and negative stool cultures	
Interventions	Both arms were treated for 4 weeks Arm 1: BID 10.0 gram sucralfate enema (N = 20) Arm 2: BID 100mg hydrocortisone enema (N = 20)	
Outcomes	Clinical improvement (at least 1 point reduction in Truelove & Witt's clinical criteria) Clinical remission ("disappearance of symptoms and endoscopic and histological signs of activity of the disease) Endoscopic improvement (at least 1 point reduction in Truelove & Witt's endoscopic criteria) Histological improvement (at least 1 point reduction in Truelove & Richard's histological criteria)	
Notes	-	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Method of sequence generation not given
Selection bias (allocation concealment)	Unclear risk	Method of allocation unclear
Performance bias	Unclear risk	Participants were not blinded
Detection bias	Low risk	Outcomes assessed by blinded investigators "using a two-physician method"
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Unclear risk	Smoking not reported in baseline characteristics

BMJ 1971		
Methods	Randomized controlled trial, 2 parallel groups, double-blind	
Participants	31 patients, mean age 41 years Diagnosis based on: endoscopy	
Interventions	Both arms were treated for 4 weeks. Arm A: OD 5mg betamethasone 17-valerate enema (N = 16) Arm B: OD 20mg prednisolone 21-phosphate enema (N = 15)	
Outcomes	Improved (not defined) Remission (absence of rectal bleeding, minimal bowel disturbance, non-haemorrhagic mucosa)	
Notes	-	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Allotment according to "a random code", but method of generation not given
Selection bias (allocation concealment)	Low risk	"Neither the doctor nor the patient knew which treatment was given"
Performance bias	Low risk	Double-blind design
Detection bias	Low risk	"[the mucosal] appearances assessed independently by two observers, one of whom was unaware of the patient's symptoms"
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Unclear risk	Smoking not reported in baseline characteristics

Campieri 1988		
Methods	Randomized controlled trial, 2 parallel arms, investigator-blind	
Participants	39 patients, mean age 91 years Diagnosis based on: endoscopy and histology performed at baseline.	
Interventions	Both arms were treated for 30 days. Arm A: OD 2.000mg 5-ASA enema (N = 20) Arm B: BID 1.000mg 5-ASA suppositories (N = 19)	
Outcomes	Clinical improvement (at least one grade reduction in Truelove & Richard's clinical score) Clinical remission (symptoms completely disappeared) Endoscopic remission (repaired rectal mucosa) Histological remission (absent signs of inflammation)	
Notes	-	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Allotment according to a random list, but method of generation not given
Selection bias (allocation concealment)	Unclear risk	Method of allocation unclear
Performance bias	Unclear risk	Participants were not blinded
Detection bias	Low risk	Outcomes assessed by blinded investigators
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Unclear risk	Smoking not reported in baseline characteristics

Campieri 1990a (Scand J Gastroenterol)	
Methods	Randomized controlled trial, 3 parallel groups, double-blind
Participants	94 patients, mean age 40 years Diagnosis based on: histology
Interventions	All arms were treated for 4 weeks. Arm A: BID 500mg 5-ASA suppositories plus OD placebo suppository (N =32) Arm B: TID 500mg 5-ASA suppositories (N = 31) Arm C: TID placebo suppositories (N = 31)
Outcomes	Clinical improvement (decrease in severity of symptoms and signs, but not meeting criteria for remission) Clinical remission (no symptoms, up to two bowel movements per day without blood) Endoscopic remission (not defined) Histological remission (not defined)
Notes	-

Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Low risk	Computerized randomization list, in blocks of 3
Selection bias (allocation concealment)	Low risk	Double-blind design
Performance bias	Low risk	Double-blind design
Detection bias	Low risk	Double-blind design
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Unclear risk	Smoking not reported in baseline characteristics

Campieri 1990b (Int J Colorect Dis)	
Methods	Randomized controlled trial, 2 parallel groups, double-blind
Participants	62 patients, mean age 36 years Diagnosis based on: clinical assessment, endoscopy, histology and negative stool cultures
Interventions	Both arms were treated for 30 days. Arm A: TID 500mg 5-ASA suppositories (N =32) Arm B: TID placebo suppositories (N = 30)
Outcomes	Clinical remission (complete disappearance of symptoms) Clinical response (reduction of at least 1 point on Truelove and Richards' scale) Endoscopic remission (healed rectal mucosa) Histological remssion (no inflammation on biopsy)
Notes	-

Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Allotment according to a random list, but method of generation not given
Selection bias (allocation concealment)	Low risk	Double-blind design
Performance bias	Low risk	Double-blind design
Detection bias	Low risk	Double-blind design
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Unclear risk	Smoking not reported in baseline characteristics

Eliakim 2007		
Methods	Randomized controlled trial, 2 parallel groups, investigator-blind	
Participants	176 patients, mean age 43 years Diagnosis based on: endoscopy, histology and negative stool cultures	
Interventions	Both arms were treated for 6 weeks Arm A: OD 2.000mg 5-ASA low-volume (60mL) foam enema (N = 91) Arm B: OD 2.000mg 5-ASA high-volume (120mL) foam enema (N = 85)	
Outcomes	Clinical improvement (at least 1 point decrease in CAI) Clinical remission (CAI equal to or lower than 4) Endoscopical remission (EI lower than 4) Mucosal healing (mucosal DAI subscore 0 or 1) Histological remission (HI equal to 1)	
Notes	-	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Low risk	Computer-generated randomization scheme
Selection bias (allocation concealment)	Unclear risk	Method of allocation unclear
Performance bias	Unclear risk	Participants were not blinded
Detection bias	Low risk	Outcomes assessed by blinded investigators
Attrition bias	Unclear risk	Used last observation carried forward, the actual amount of imputed data is not reported
Reporting bias	Low risk	-
Other bias	Low risk	-

Farup 1995		
Methods	Randomized controlled trial, 2 parallel groups, open label	
Participants	50 patients, mean age 43 years Diagnosis based on: endoscopy performed at baseline	
Interventions	Both arms were treated for 4 weeks Arm A: BID 500mg 5-ASA suppositories (N = 24) Arm B: BID 178mg hydrocortisone foam enemas (N = 26)	
Outcomes	Partial responder (any decrease in DAI, but not meeting criteria for complete responder) Complete responder (DAI equal to or lower than 2) Histological improvement (not defined)	
Notes	-	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Method of sequence generation not given
Selection bias (allocation concealment)	High risk	Open-label design
Performance bias	High risk	Open-label design
Detection bias	High risk	Open-label design
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Low risk	Smoking not reported in baseline characteristics

Gionchetti 1997		
Methods	Randomized controlled trial, 2 parallel groups, investigator-blind	
Participants	50 patients, mean age 39 years Diagnosis based on: endoscopy and histology	
Interventions	Both arms were treated for 1 month Arm A: OD 1.000mg 5-ASA suppository (Pentasa ®) (N =25) Arm B: BID 500mg 5-ASA suppositories (Claversal ®) (N = 25)	
Outcomes	Clinical remission (sum of clinical DAI subscores = 0) Endoscopic remission (endoscopy DAI subscore = 0) Histological remission (histological activity index = 0)	
Notes	-	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Low risk	Computer predetermined randomization list
Selection bias (allocation concealment)	Unclear risk	Method of allocation unclear
Performance bias	Unclear risk	Participants were not blinded
Detection bias	Low risk	Outcomes assessed by blinded investigators
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Unclear risk	Smoking not reported in baseline characteristics

Gionchetti 1998		
Methods	Randomized controlled trial, 2 parallel groups, investigator-blind	
Participants	58 patients, mean age 35 years Diagnosis based on: previously confirmed diagnosis	
Interventions	Both arms were treated for 4 weeks Arm A: TID 800mg 5-ASA tablets (N =29) Arm B: TID 400mg 5-ASA suppositories (N = 29)	
Outcomes	Clinical remission (sum of clinical DAI subscores = 0) Endoscopic remission (mucosal DAI subscore = 0) Histological remission (grade 1 in Truelove & Richard's criteria)	
Notes	-	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Low risk	Computer predetermined randomization list
Selection bias (allocation concealment)	Unclear risk	Method of allocation unclear
Performance bias	Unclear risk	Participants were not blinded
Detection bias	Low risk	Outcomes assessed by blinded investigators
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Unclear risk	Smoking not reported in baseline characteristics

Gross 2006	
Methods	Randomized controlled trial, 2 parallel groups, double-blind
Participants	204 patients, mean age 44 years Diagnosis based on: endoscopy, histology and negative stool culture
Interventions	Both arms were treated for 4 weeks Arm A: OD 2mg budesonide foam enema plus OD placebo liquid enema (N = 105) Arm B: OD 2mg budesonide liquid enema plus OD placebo foam enema (N = 105)
Outcomes	Clinical remission (CAI equal to or lower than 4) Clinical improvement based on CAI (no definition given) Change in DAI Clinical remission and improvement based on DAI (no definition given) Endoscopic remission and improvement (no definition given) Histological improvement (no definition given)
Notes	-

Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Method of sequence generation not given
Selection bias (allocation concealment)	Low risk	Double-blind design
Performance bias	Low risk	Double-blind design
Detection bias	Low risk	Double-blind design
Attrition bias	Unclear risk	Used last observation carried forward, the actual amount of imputed data is not reported
Reporting bias	Low risk	-
Other bias	Low risk	-

Hanauer 2005	
Methods	Randomized controlled trial, 2 parallel groups, double-blind
Participants	40 patients, mean age 42 years Diagnosis based on: previous diagnosis
Interventions	Both arms were treated for 6 weeks Arm A: TID 800mg 5-ASA plus TID 2 placebo tablets (N = 20) Arm B: TID 1.600mg 5-ASA plus TID 2 placebo tablets (N = 20)
Outcomes	Complete remission: normal stool frequency, no rectal bleeding, good PFA and PGA score, normal endoscopy findings
Notes	-

Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Method of sequence generation not given
Selection bias (allocation concealment)	Low risk	Double-blind design
Performance bias	Low risk	Double-blind design
Detection bias	Low risk	Double-blind design
Attrition bias	Unclear risk	Used last observation carried forward, the actual amount of imputed data is not reported
Reporting bias	Low risk	-
Other bias	Low risk	-

Larnet 2005		
Methods	Randomized controlled trial, 2 parallel groups, open label	
Participants	87 patients, mean age 39 years Diagnosis based on: endoscopy	
Interventions	Both arms were treated for 6 weeks Arm A: BID 500mg 5-ASA suppositories (N = 48) Arm B: OD 1.0000mg 5-ASA suppository (N = 39)	
Outcomes	Clinical remission (comparing DAI week 3 with DAI week 6) Improvement in each of 4 DAI subscales	
Notes	-	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Low risk	Randomization list generated by an automated number program
Selection bias (allocation concealment)	High risk	Assignment in blocks of 5, but in an open label design
Performance bias	High risk	Open label design
Detection bias	High risk	Open label design
Attrition bias	Unclear risk	Used last observation carried forward, the actual amount of imputed data is not reported
Reporting bias	Low risk	-
Other bias	Low risk	-

Pokrotnieks 2000		
Methods	Randomized controlled trial, 2 parallel groups, double-blind	
Participants	111 patients, mean age 44 years Diagnosis based on: endoscopy, histology and stool culture	
Interventions	Both arms were treated for 6 weeks Arm A: OD 2.000mg 5-ASAfoam enema (N = 54) Arm B: OD placebo foam enema (N = 57)	
Outcomes	Clinical remission (Rachmilewitz' CDAI equal to or lower than 4, with at least 2 point decrease from baseline) Endoscopic remission (EI lower than 4) Histological improvement (At least 1 point reduction in Floren's histological index)	
Notes	In "Methods" section, clinical remission is defined, but in "Results"section the terms "clinical response" and "clinical remission" are used interchangeably	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Low risk	Lists generated by the SAS program 'Random'
Selection bias (allocation concealment)	Low risk	Double-blind design
Performance bias	Low risk	Double-blind design
Detection bias	Low risk	Double-blind design
Attrition bias	Unclear risk	Used last observation carried forward, the actual amount of imputed data is not reported
Reporting bias	Low risk	-
Other bias	Low risk	-

van Hees 1980		
Methods	Randomized controlled trial, 3 parallel groups, double-blind	
Participants	45 patients (33 unique patients), mean age 37 years Diagnosis based on: endoscopy	
Interventions	All arms were treated for 4 weeks Arm A: BID 200mg 5-ASA suppositories (N = 15) Arm B: BID 300mg sulphapyridine suppositories (N = 15) Arm C: BID placebo suppositories (N = 15)	
Outcomes	Successful treatment (complete disappearance of clinical symptoms and a normal rectal mucosa at sigmoidoscopy)	
Notes	12 patients were allowed to participate in the study twice	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Method of sequence generation not given
Selection bias (allocation concealment)	Low risk	Double-blind design
Performance bias	Low risk	Double-blind design
Detection bias	Low risk	Double-blind design
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	High risk	12 patients were enrolled in the study twice, smoking not reported in baseline characteristics

van Hogezand 1988		
Methods	Randomized controlled trial, 2 parallel groups, double-blind	
Participants	34 patients, mean age 39 years Diagnosis based on: endoscopy	
Interventions	Both arms were treated for 4 weeks Arm A: BID 300mg 5-ASA suppositories (N = 18) Arm B: BID 300mg acetyl-5-ASA suppositories (N = 16)	
Outcomes	Successful treatment (complete disappearance of clinical symptoms and a normal rectal mucosa at sigmoidoscopy)	
Notes	-	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Method of sequence generation not given
Selection bias (allocation concealment)	Low risk	Minimization with a random component was used for allocation
Performance bias	Low risk	Double-blind design
Detection bias	Low risk	Double-blind design
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Low risk	-

Watanabe 2013		
Methods	Randomized controlled trial, 2 parallel groups, double-blind	
Participants	129 patients, mean age 41 years	
	Diagnosis based on: endoscopy and histology performed at baseline	
Interventions	Both arms were treated for 4 weeks	
	Arm A: OD 1.000mg 5-ASA suppository (N = 65)	
	Arm B: OD placebo suppository (N = 64)	
Outcomes	Clinical remission (UC-DAI of 2 or less, with bleeding subscore = 0)	
	Endoscopic remission (endoscopic score of 0 or 1)	
Notes	-	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Computer generated randomization scheme
Selection bias (allocation concealment)	Low risk	Dynamic assignment
Performance bias	Low risk	Double-blind design
Detection bias	Low risk	Double-blind design
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Low risk	-

Williams 1987		
Methods	Randomized controlled trial, 2 parallel groups, double-blind	
Participants	27 patients, mean age 40 years	
	Diagnosis based on: endoscopy and histology performed at baseline	
Interventions	Both arms were treated for 6 weeks	
	Arm A: TID 500mg 5-ASA suppositories (N = 14)	
	Arm B: TID placebo suppositories (N = 13)	
Outcomes	Remission (DAI = 0)	
Notes	-	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Method of sequence generation not given
Selection bias (allocation concealment)	Low risk	Double-blind design
Performance bias	Low risk	Double-blind design
Detection bias	Low risk	Double-blind design
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Unclear risk	Smoking not reported in baseline characteristics

d'Albasio 1998	
Methods	Randomized controlled trial, 3 parallel groups, double-blind
Participants	111 patients, mean age 40 years Diagnosis based on: endoscopy and histology
Interventions	All arms were treated for 12 months Arm A: BID 500mg 5-ASA suppositories (N=36) Arm B: OD 500mg 5-ASA suppository plus OD placebo suppository (N = 40) Arm C: BID placebo suppositories (N = 35)
Outcomes	Clinical remission (up to two bowel movements per day without blood) Endoscopic improvement (grade 0 or 1 on Baron's endoscopic criteria) Histological improvement (grade 0 or 1 in Truelove & Richard's histological criteria)
Notes	-

Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Method of sequence generation not given
Selection bias (allocation concealment)	Low risk	Double-blind design, allocation stratified per center
Performance bias	Low risk	Double-blind design
Detection bias	Low risk	Double-blind design
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Unclear risk	Smoking not reported in baseline characteristics

d'Arienzo 1990	
Methods	Randomized controlled trial, 2 parallel groups, double-blind
Participants	17 patients, mean age 40 years Diagnosis based on: previously documented proctitis
Interventions	Both arms were treated for 12 months Arm A: BID 400mg 5-ASA suppositories (N=9) Arm B: BID placebo suppositories (N = 8)
Outcomes	Clinical remission (absence of blood in stools, no symptoms) Endoscopic remission (endoscopic severity grade 0 or 1 in Blackstone's scoring criteria) Histological remission (histological severity grade 0 or 1 in Friedman's criteria)
Notes	-

Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Low risk	Random number table
Selection bias (allocation concealment)	Low risk	Double-blind design
Performance bias	Low risk	Double-blind design
Detection bias	Low risk	Double-blind design
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Unclear risk	Smoking not reported in baseline characteristics

Hanauer 2000		
Methods	Randomized controlled trial, 2 parallel groups, double-blind	
Participants	65 patients, mean age 38 years Diagnosis based on: previous diagnosis	
Interventions	Both arms were treated for 12 to 24 months Arm A: OD 500mg 5-ASA suppository (N = 31) Arm A: OD placebo suppository (N = 35)	
Outcomes	Relapse (rectal bleeding or increased stool frequency for more than 1 week and endoscopic inflammation)	
Notes	-	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Method of sequence generation not given
Selection bias (allocation concealment)	Low risk	Double-blind design
Performance bias	Low risk	Double-blind design
Detection bias	Low risk	Double-blind design
Attrition bias	Unclear risk	Missing data only mentioned in one table, unclear if there is more missing data
Reporting bias	Low risk	-
Other bias	Low risk	-

Nilsson 1995		
Methods	Randomized controlled trial, 2 parallel groups, double-blind	
Participants	69 patients, mean age 42 years Diagnosis based on: previous diagnosis	
Interventions	Both arms were treated for 6 to 18 months Arm A: BID 500mg olsalazine capsule plus BID placebo capsule (N = 37) Arm B: BID 1.000mg SASP capsule plus BID placebo capsule (N = 32)	
Outcomes	Relapse (clinical complaints, confirmed endoscopically)	
Notes	-	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Method of sequence generation not given
Selection bias (allocation concealment)	Low risk	Double-blind design
Performance bias	Low risk	Double-blind design
Detection bias	Low risk	Double-blind design
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Low risk	-

Travis 1994		
Methods	Randomized controlled trial, 3 parallel groups, patient-blind*	
Participants	26 patients, mean age 48 years Diagnosis based on: clinical, endoscopic, histological criteria	
Interventions	All arms were treated for 12 months Arm A: OD daily 500mg olsalazine tablet plus BID placebo tablets (N = 8) Arm B: BID 500mg olsalazine tablets plus OD daily placebo tablet (N = 8) Arm C: TID 500mg olsalazine tablets (N = 10)	
Outcomes	Relapse (increase in bowel frequency with blood or mucus and evidence of active disease on sigmoidoscopy)	
Notes	*Placebo tablets were used, making blinding of patients likely, but blinding of investigators is not mentioned	
Risk of bias table		
Bias type	Authors' judgment	Support for judgment
Selection bias (sequence generation)	Unclear risk	Method of sequence generation not given
Selection bias (allocation concealment)	Unclear risk	Method of allocation unclear
Performance bias	Unclear risk	Blinding of investigators unclear
Detection bias	Unclear risk	Blinding of investigators unclear
Attrition bias	Unclear risk	Missing data not reported
Reporting bias	Low risk	-
Other bias	Unclear risk	Smoking not reported in baseline characteristics

Description of included studies

Remission induction

18 studies (N=1546) were remission induction studies[34-51]. Of the 18 induction studies, 15 involved 5-ASA and 6 were placebo controlled[38-39,47-48,50-51], with one study also examining topical SASP in a third treatment arm[48]. 5 studies compared different topical 5-ASA doses or formulations[34,37,40,42,46], 3 studies reported on topical 5-ASA versus hydrocortisone foam, oral 5-ASA or topical acetyl-5-ASA[41,43,49]. One study compared different oral 5-ASA doses[45]. The remaining 3 induction studies assessed topical application of sucralfate and hydrocortisone, betamethasone and prednisolone and budesonide foam and budesonide enema[35,36,44].

10 studies were conducted in double blind fashion[36,38-39,44-45,47-51], 6 were investigator-blind[34-35,37,40,42-43] and 2 studies were open-label[41,46].

Maintenance of remission

Five studies (N=288) were maintenance of remission studies[52-56]. 3 studies concerned topical 5-ASA[52-54], all three of which were placebo controlled studies. The two remaining trials examined SASP and olsalazine[55-56].

The exact blinding in one study was unclear[56]. The manuscript states the use of placebo, thus blindness at the patient level was deemed likely. However, the blinding of the investigators was not specifically reported. The remaining studies were double-blind trials.

Description of participants

All patients included were adults, and 46% were females. All included patients had a diagnosis of UC, either previously established or established at enrollment through at least clinical presentation and endoscopic assessment. Two studies[48-49], studied patients with "idiopathic proctitis", but after detailed review of the studies it was judged that these patients had UP. As such, the patients in these studies were considered eligible for this review.

Description of interventions

Remission induction

In the 6 trials comparing topical 5-ASA with placebo, the daily dose ranged from 400mg to 2.000mg, with 5 studies examining suppositories[38-39,48,50-51] and one study examining foam enema[45]. One study had a third treatment arm with differently dosed 5-ASA[38], another had a third treatment arm with SASP in a daily dose of 600mg[48].

5 studies compared different doses or concentrations of topical 5-ASA, ranging from 1.000mg to 2.000mg. 3 studies compared suppositories[34,42,46] one compared suppository with liquid enema[37] and one compared low volume (60mL) with high volume (120mL) foam enemas[40].

3 studies reported on daily doses of 600mg to 1.200mg topical 5-ASA versus 356mg hydrocortisone foam, 2.400mg oral 5-ASA and 850mg acetyl-5-ASA[41,43,49].

One study compared 2.400mg daily 5-ASA with 4.800mg daily 5-ASA[45].

The remaining 3 induction studies compared topical application of 20 gram sucralfate with 200mg hydrocortisone, 5mg betamethasone with 20mg prednisolone and 2mg budesonide foam with 2mg budesonide liquid enema[35-36,44].

The treatment duration was 4 weeks in 13 studies[35-39,41-44,47-50] and 6 weeks in 5 studies[34,40,45-46,51].

Maintenance of remission

The 3 placebo controlled studies investigated topical 5-ASA in daily doses ranging from 500mg to 1.000mg[52-54]. The two other studies compared different doses of oral SASP or olsalazine, one study in two arms comparing 1.000mg SASP with 2.000mg olsalazine[55], the other in three arms with 500mg, 1.00mg and 2.000mg olsalazine[56].

Treatment and follow-up duration varied from at least 6 months in one study[55] to 12 months in 3 studies[52-53,56] and 24 months in the remaining trial[54].

Risk of bias in included studies

The summary of the risk of bias assessments is provided in Supplemental Figure S2.1. Two studies were considered at high risk of bias because of lack of blinding[41,46]. The remaining studies were of acceptable to high methodological quality. All studies were self-reported as randomized controlled trials, but only 8 reported their method of random sequence generation[36,38,40,42-43,45-47,50,53]. Similarly, allocation concealment was described in only 13 studies[36,38-39,44-45,47,50,52-54]. It was difficult to assess attrition bias, no studies reported the total amount of missing data, though 6 studies used "last observation carried forward" to deal with missing data[34,40,44-47]. The risk of reporting bias was considered low, as no studies reported post-hoc analyses. As for other forms of bias and confounding, in studies published before 2000, smoking behavior is not reported, which may have confounded their results. Additionally, one study enrolled and randomized 12 of 33 patients twice, which may have influenced the results[48].

Effects of interventions – Primary outcome

Remission induction

The results of the pooled analyses are shown in Figure 2.3.

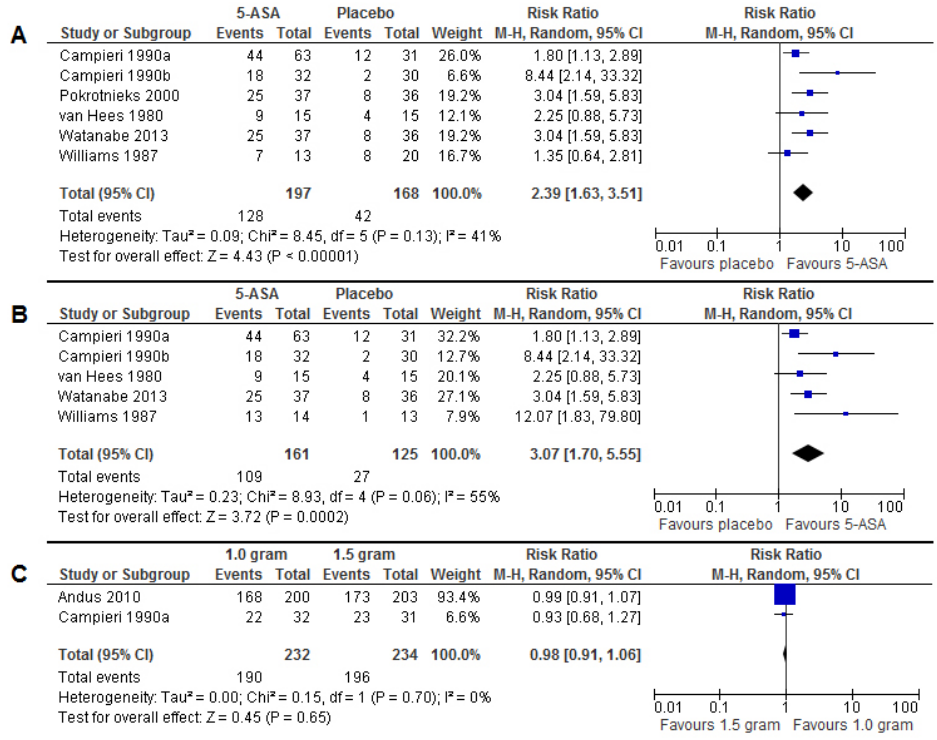

Figure 2.3 Comparison: topical 5-ASA versus placebo, induction of clinical remission. Panel A: Forest plot of clinical remission, for all topical 5-ASA formulations and doses compared with placebo; Panel B: Forest plot of clinical remission, for all 5-ASA suppositories compared with placebo; Panel C: Forest plot of clinical remission, for 500mg 5-ASA suppositories BID compared with TID.

Topical 5-ASA versus placebo

Six trials (N=365) examined topical 5-ASA (suppository or enema) versus placebo[38-39,47-48,50-51]. Patients treated with 5-ASA achieved clinical remission in 65.0%, whereas those treated with placebo achieved remission in 25.0%. The pooled relative risk (RR) of achieving clinical remission for all trials was 2.39 (95% CI 1.63-3.51, I2=41%, P<0.00001) using a random-effects model (Figure 2.3a). The suppository only subgroup (N=286) was analyzed after removing the single study with foam enema[48], resulting in a 67.7% clinical remission rate for 5-ASA compared with a 21.1% rate for placebo. The pooled RR of achieving clinical remission for this subgroup was 3.07 (95% CI 1.70-5.55, I2=55%, P=0.0002) using a random-effects model (Figure 2.3b).

Topical 5-ASA, different doses

Only comparison of different daily topical 5-ASA doses could be performed, regarding 1.000mg versus 1.500mg suppositories. Two studies (N=466) reported on BID 500mg suppositories versus TID 500mg suppositories[34,38]. Reported clinical remission rates were 81.9% and 83.8% for the BID and TID groups respectively. The pooled RR of achieving clinical remission for BID compared with TID was 0.98 (95% CI 0.91-1.06, I2=0%, P=0.65) using a random-effects model (Figure 2.3c).

Topical 5-ASA, same dose, different formulation

One study (N=39) compared a daily dose of 2.000mg 5-ASA in suppository formulation with enemas[37]. This study found clinical remission rates of 78.9% and 80.0% for the suppository and enema groups respectively. This difference was not statistically significant.

Another trial (N=176) compared liquid enemas with foam enemas, both at a daily dose of 2.000mg[40]. With remission rates of 78.0% and 75,2% for the foam and liquid groups respectively, the study found no statistically significant difference.

One trial (N=50) compared Pentasa® with Claversal® suppositories, both in daily dose of 1.000mg[42]. No statistically significant difference was found, with remission rates of 84.0% and 76.0% in the Pentasa® and Claversal® groups respectively.

Finally, one study (N=87) compared BID 500mg suppositories with OD 1.000mg suppositories[46]. This study reported remission rates of 43.8% and 59.0%, which was not statistically significantly different.

5-ASA, other comparisons

One trial (N=58) compared a daily dose of 2.400mg oral 5-ASA with 1.200mg 5-ASA suppositories[43]. The clinical remission rates were 41.4% versus 89.6% respectively, statistically significant (P<0.01) in favor of topical treatment.

Another study (N=40) compared 2.400mg daily oral 5-ASA with 4.800mg daily oral 5-ASA[45]. No statistically significant difference was found, as the groups achieved remission in 20.0% and 30.0% respectively.

One study (N=40) compared a daily dose of 1.000mg 5-ASA suppositories with a daily dose of 356mg hydrocortisone foam enema[41]. Clinical remission rates were 58.3% and 34.6% respectively, which is a non-significant difference.

Another trial (N=30) compared a daily dose of 400mg 5-ASA suppositories with 600mg sulphapyridine suppositories[48]. This study found a significant difference in favor of 5-ASA (P=0.02), with remission rates of 60.0% compared with 13,3% for the 5-ASA and sulphapyridine groups respectively.

The last trial (N=34) compared a daily dose of 400mg 5-ASA suppositories with 850mg acetyl-5-ASA suppositories[49]. It found clinical remission rates of 61.1% and 18.8% respectively. This result was statistically significant (P=0.03) in favor of 5-ASA.

Other treatments

One study (N=40) compared a daily dose of 20 gram sucralfate enemas with 200mg hydrocortisone enemas[35]. The reported clinical remission rates were 15.0% and 42.1% respectively. This difference was not significant.

Another study (N=31) compared OD 5mg betamethasone enema with OD 20mg prednisolone enema[36]. The study reports clinical remission rates of 55.0% and 44.0% respectively, which is not a statistically significant difference.

Finally, one trial (N=204) compared 2mg budesonide foam enema with 2mg budesonide liquid enema[44]. The reported clinical remission rates were 58.1% and 68.7% respectively. This difference was not statistically significant.

Maintenance of remission

The results of the pooled analyses are shown in Figure 2.4.

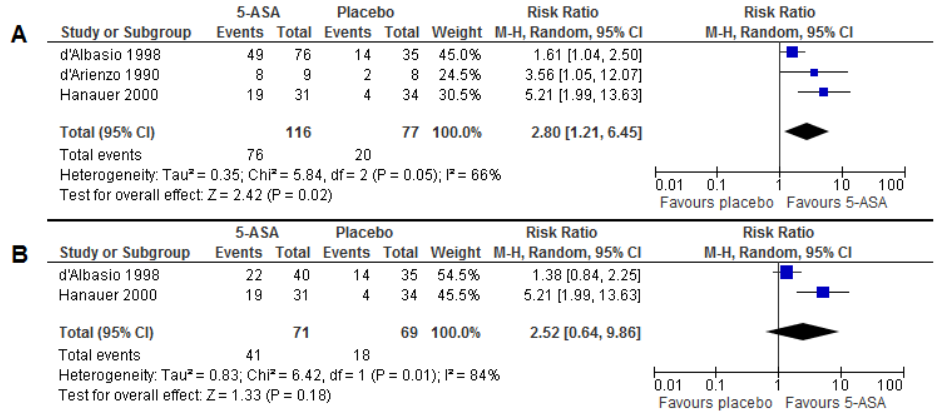

Figure 2.4 Comparison: topical 5-ASA versus placebo, maintenance of clinical remission. Panel A: Forest plot maintained clinical remission, for all 5-ASA suppositories compared with placebo; Panel B: Forest plot maintained clinical remission, for 500mg 5-ASA suppositories compared with placebo.

Topical 5-ASA versus placebo

Three trials (N=193) examined topical 5-ASA (suppositories, daily dose 500mg to 1.000mg) versus placebo[52-54]. Patients treated with 5-ASA maintained clinical remission in 65.5%, but in patients receiving placebo only 26.0% maintained their remission. The pooled RR of maintaining clinical remission for all trials was 2.80 (95% CI 1.21-6.45, I2=66%, P=0.02) using a random-effects model (Figure 2.4a). A subgroup analysis (N = 140) of the studies[52,54] examining 500mg 5-ASA suppositories showed a 57.7% maintained clinical remission rate for 5-ASA compared with 26.1% for placebo. The pooled RR of maintaining clinical remission with this dose of 5-ASA was 2.52 (95% CI 0.64 – 9.86, I2=85%, P=0.18) using a random-effects model (Figure 2.4b).

Topical 5-ASA, different doses

One trial (N=76) compared OD 500mg 5-ASA suppositories with BID 500mg suppositories[52]. The maintained clinical remission rates were 72.5% and 91.7% respectively, a statistically significant difference in favor of the higher 5-ASA dose (P=0.03).

Oral SASP and olsalazine

One study (N=69) compared a daily dose of 1.000mg oral SASP with 2.000mg oral olsalazine[55]. In this study, remission was maintained in 69.0% and 58.1% for the SASP

and olsalazine groups respectively, though this difference was not statistically significant.

The other trial (N=26) compared three different daily doses of olsalazine, 500mg, 1.000mg and 2.000mg[56]. In these small groups, maintained remission rates were 50.0%, 62.8% and 90% for the 500mg, 1.000mg and 2.000mg groups respectively. The between group differences were not statistically significant.

Effects of interventions – Secondary outcomes

Remission induction

Topical 5-ASA versus placebo

The results of the pooled analyses are shown in Figure 2.5.

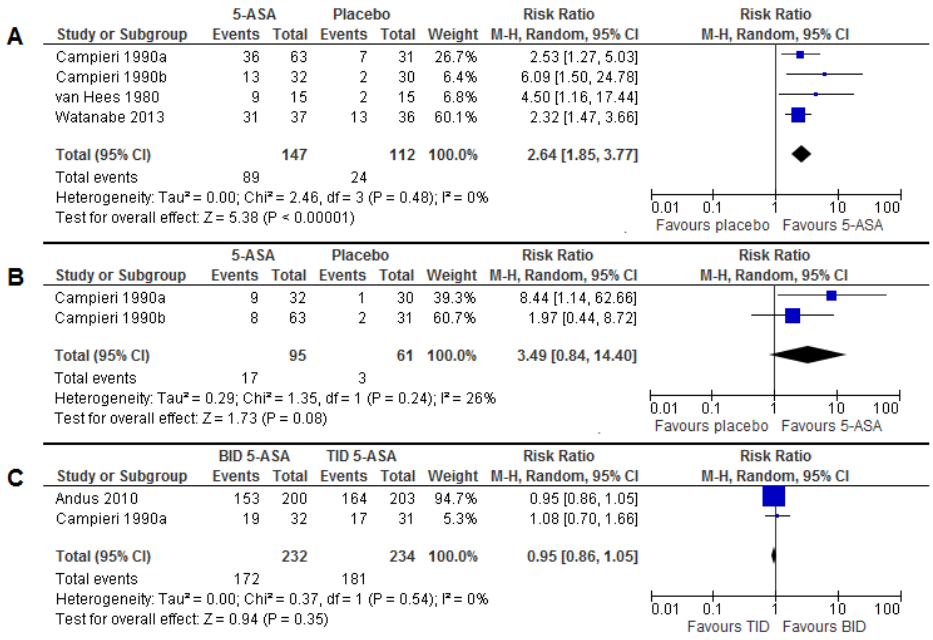

Figure 2.5 Comparison: topical 5-ASA versus placebo, induction of endoscopic and histological remission. Panel A: Forest plot of endoscopic remission, for all 5-ASA suppositories compared with placebo; Panel B: Forest plot of histological remission, for all 5-ASA suppositories compared with placebo; Panel C: Forest plot of endoscopic remission, for 500mg 5-ASA suppositories BID compared with TID.

Endoscopic remission rates were reported by 4 of 6 placebo controlled studies[38-39,48,50], all of which studied 5-ASA suppositories. Interestingly, the endoscopic remission rates in one study[50] were higher than the clinical remission rates. These studies (N=259) found endoscopic remission rates of 60.5% and 21.4% for 5-ASA and placebo respectively. The pooled RR of achieving clinical remission for all trials was 2.64 (95% CI 1.85-3.77, I2=0%, P<0.00001) using a random-effects model (Figure 2.5a).

Histological remission rates were reported (N=156) by two studies[38-39]. The histological remission rates were 17.9% and 4.93% for the 5-ASA and the placebo groups respectively. The pooled RR of achieving histological remission for all trials was 3.49 (95% CI 0.84-14.40, I2=26%, P=0.08) using a random-effects model (Figure 2.5b).

Topical 5-ASA, different doses

Two studies (N=466) reported on endoscopic remission rates, when comparing BID 500mg suppositories with TID 500mg 5-ASA suppositories[34,38]. The endoscopic remission rates were 74.1% and 77.4% for the BID and TID groups respectively. The pooled RR of achieving clinical remission for all trials was 0.95 (95% CI 0.86-1.05, I2=0%, P=0.35) using a random-effects model (Figure 2.5c).

Histological remission rates (N=403) were reported only by one of these studies[34]. The reported remission rates were of 41.5% and 44.8%, a non-significant difference.

Topical 5-ASA, same dose, different formulation

Both endoscopic and histological remission rates were reported by the single study (N=39) that compared a daily dose of 2.000mg 5-ASA in suppository formulation with the same dose in enema formulation[37]. Endoscopic remission rates were 73.7% and 65.0% whilst histological remission rates were 63.2% and 45.0% for the suppository and enema groups respectively. For both comparisons, the difference was not statistically significant.

The study (N=50) comparing Pentasa® with Claversal® suppositories, both in daily dose of 1.000mg, reported both endoscopic and histological remission rates[42]. No statistically significant difference was found, with endoscopic remission rates of 80.0% and 72.0% and histological remission in 52.0% and 48.0% in the Pentasa® and Claversal® groups respectively.

The remaining studies that reported on different formulations or concentrations of 5-ASA in the same dose, either did not report endoscopic or histological remission rates[46], or did not specifically report those outcomes for patients with UP[40].

5-ASA, other comparisons

The study (N=58) comparing 2.400mg oral 5-ASA with 1.200mg 5-ASA suppositories reported both endoscopic and histological remission rates[43]. Both rates were significantly (P<0.01) in favor of topical treatment, with endoscopic remission rates of 51.7% and 13.8% and histological remission rates of 37.9% and 6.8%.

The trial (N=30) that studied 400mg 5-ASA suppositories and 600mg sulphapyridine suppositories only reported on endoscopic remission[48]. This study found a significant difference in favor of 5-ASA (P=0.02), with remission rates of 60.0% compared with 13.3% for the 5-ASA and sulphapyridine groups respectively.

The study (N=34) that compared a daily dose of 400mg 5-ASA suppositories with 850mg acetyl-5-ASA suppositories reported only endoscopic remission rates[49]. It found endoscopic remission rates of 66.7% and 12.5% respectively. This result was statistically significant (P=0.005) in favor of 5-ASA.

The remaining studies concerning 5-ASA did not report endoscopic or histological remission rates[41,45].

Other treatments

The single study (N=40) comparing 20 gram sucralfate enemas with 200mg hydrocortisone enemas did report both endoscopic and histological remission rates[35]. Endoscopic remission was found in remission rates were 30.0% and 35.0%, whilst histological remission was seen in 20.0% and 30.0% for the sucralfate and hydrocortisone groups respectively. For both comparisons, the difference did not reach statistical significance.

The remaining studies either did not report endoscopic or histological remission rates[36], or did not report those outcomes specifically for patients with UP[44].

Maintenance of remission

Topical 5-ASA versus placebo

None of the 3 maintenance studies comparing topical 5-ASA with placebo, reported on endoscopic or histological remission. Only one study reported the mean times to relapse[54], which were 453.4 days and 158.0 days for the 5-ASA and placebo groups respectively.

Topical 5-ASA, different doses

The one trial comparing OD 500mg 5-ASA suppositories with BID 500mg suppositories did not report on endoscopic remission, histological remission or time to relapse[52].

Oral SASP and olsalazine

Neither of the trials examining SASP or olsalazine reported on endoscopic remission, histological remission or time to relapse[55-56].

Discussion

The objectives of this review were to assess the effects on inducing or maintaining clinical remission of different types of drug therapies for ulcerative proctitis. To our knowledge, no other reviews on drug therapy for ulcerative proctitis have been published. This review included 23 studies involving 1833 patients. The majority of studies examined drug efficacy for remission of induction. As most studies employed different clinical scoring systems to define clinical remission, endoscopic and histological remission were secondarily assessed, as they are considered less ambiguous. However, most studies were of a short duration, possibly resulting in too limited follow-up time to achieve mucosal healing, thus possibly underestimating the endoscopic response rates. Despite these limitations, we feel that the data allows for two firm conclusions to be drawn.

Firstly, the effectiveness of topical 5-ASA for inducing remission is confirmed. The ability of topical 5-ASA to induce clinical remission was clearly shown in several placebo controlled studies. Patients receiving topical 5-ASA were 2.39 times more likely to achieve clinical remission than those receiving placebo. No clear dose response relationship was found between topical 5-ASA and clinical remission, though only two studies compared different 5-ASA doses. Additionally, endoscopic evaluations in

4 studies showed a clear benefit of topical 5-ASA over placebo. Histological remission rates were assessed in only two studies and showed no clear benefit of topical 5-ASA over placebo.

Secondly, topical 5-ASA was more effective at maintaining clinical remission than placebo, as shown in 3 studies, with a relative risk of maintaining remission of 2.80 for 5-ASA.

All other studies included in this review examined unique combinations of drugs and comparators, precluding us from performing additional pooled analyses. As such, no new conclusions can be drawn from this review in regards to the drugs studied in those trials. Also, no trials investigating the efficacy of thiopurines or anti-TNF in patients with UP were identified by the systematic search.

In summary, this systematic review and meta-analysis supports the use of topical 5-ASA as the first treatment in UP. No recommendations for UP patients failing 5-ASA is provided by this review, as there are no randomized controlled trials in UP patients examining the efficacy of other drug therapies. This should be the focus of future research.

Authors' conclusions

Implications for practice

The role of topical 5-ASA as first line treatment for UP has been confirmed in this review, both for induction and maintenance of remission. Additionally, there is limited evidence available for the superiority of topical 5-ASA compared with oral 5-ASA in UP. As such, this review will not change the first line treatment for UP patients.

Due to the paucity of studies examining other drugs than 5-ASA, this review cannot draw any conclusions on which drug is the most optimal second line therapy in UP. Similarly, this review cannot reliably assess the efficacy of corticosteroids, thiopurines or anti-TNF alpha therapy in UP patients.

Implications for research

More trials comparing topical 5-ASA with placebo for induction of clinical remission in UP do not appear to be justified. Given the lack of studies examining therapies other

than 5-ASA, future trials should investigate other drug therapies for UP patients. Given the current step-up method of treatment, it may be valuable to perform these studies mainly in 5-ASA therapy refractory patients.

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Supplemental material

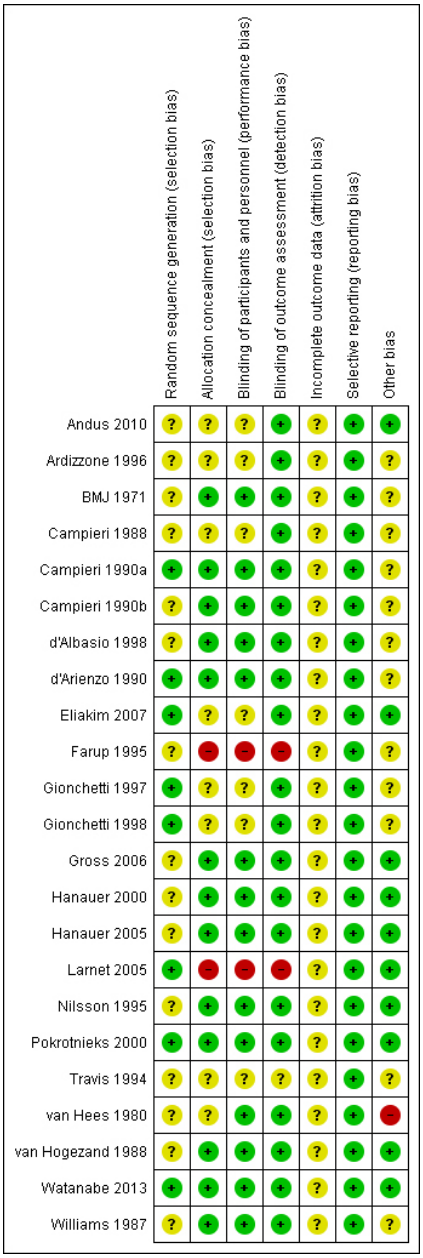

Figure S2.1

Chapter 3

Efficacy and safety of tacrolimus vs beclomethasone suppositories in patients with refractory ulcerative proctitis in a randomized trial

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Abstract

Background & Aims

Ulcerative proctitis (UP) refractory to 5-aminosalicylic acid (5-ASA) suppositories is a challenge to treat, often requiring step up to immunomodulator or biological therapy. Topical tacrolimus is effective and safe in patients with refractory UP. However, it is not clear how tacrolimus suppositories fit into in the treatment algorithm of UP.

Methods

We performed a randomized controlled, double-blind study at 8 hospitals in The Netherlands and Belgium from 2014 through 2017. Eighty-five patients with refractory UP (65% women) were randomly assigned to groups given once daily tacrolimus suppositories (2mg, n=43) or beclomethasone (3mg, n=42) for 4 weeks. The primary outcome was clinical response (decrease in Mayo score of 3 or more). Secondary outcomes included clinical remission, endoscopic response and remission, adverse events and quality of life. Outcomes were compared using Fisher's exact test and Mann-Whitney U test.

Results

Proportions of patients with clinical responses were 63% in the tacrolimus group and 59% in the beclomethasone group (P=0.812); proportions of patients in clinical remission were 46% and 38%, respectively (P=0.638). Proportions of patients with an endoscopic response were 68% and 60% in the tacrolimus group and in the beclomethasone group (P=0.636); proportions in endoscopic remission rates were 30% and 13%, respectively (P=0.092) Median increases in the inflammatory bowel disease questionnaire score were 18.0 in the tacrolimus group and 20.5 in the beclomethasone group (P=0.395). Adverse event rates did not differ significantly between groups.

Conclusions

In a 4-week randomized controlled trial, tacrolimus and beclomethasone suppositories induce comparable clinical and endoscopic responses in patients with UP refractory to 5-ASA. There were no significant differences in adverse events rates. Tacrolimus and beclomethasone suppositories are therefore each safe and effective treatment options for 5-ASA refractory disease.EUDRACT 2013-001259-11; Netherlands Trial Register NL4205 / NTR4416.

Introduction

Up to 40% of newly diagnosed ulcerative colitis patients have disease limited to the rectum and are considered incident cases of ulcerative proctitis (UP)[1]. Adequate therapy for UP may not only be important for symptom control and quality of life[2] but may also reduce the risk of progression of disease extent. Epidemiological and retrospective studies have shown that in patients with UP progression of disease extent occurs in up to 50% of patients[3,4], whilst in retrospective studies the risk of progression appears to be higher in patients with persistent or recurrent disease activity[5].

The first step in the current treatment scheme for UP consists of topical 5-ASA therapy, usually in suppository form. Though 5-ASA has a remission induction rate of 65%[6], maintenance of remission occurs in only 50% of patients. Current guidelines advise locally administered corticosteroids in these refractory patients[7], though this therapy induces remission in only 46% of patients[8] and comes with risks of systemic side effects such as suppression of the hypothalamic-pituitary-adrenal axis[9].

When UP is refractory to both 5-ASA and corticosteroids, step-up to systemically administered immunosuppressive drugs such as thiopurines and biologicals is recommended in the guidelines[7]. However, robust data regarding these drugs in UP is lacking, as patients with UP are usually excluded from clinical trials. Furthermore the use of these systemically administered drugs might be associated with side effects and higher costs, particularly in the case of biological therapies[10]. Therefore, a proven effective topical therapy will expand the current therapeutic possibilities.

Systemically applied calcineurin inhibitors such as ciclosporin and tacrolimus are already established therapeutic options for steroid refractory UC[7]. Several pilot studies have shown that topical tacrolimus is a safe and effective induction therapy in refractory UP, with clinical response rates up to 80%[11,12]. Recently the results of a double-blind, randomized controlled trial were published, showing highly significant differences between rectal tacrolimus and placebo at the interim analysis[13]. The response and remission rates were similar to the pilot studies, further reinforcing the basis of this study. These studies formed the basis for this randomized controlled trial comparing tacrolimus suppositories with beclomethasone suppositories for the treatment of patients with 5-ASA refractory UP.

Materials and methods

Study Design

A randomized controlled, double-blind multicenter study was performed in 8 hospitals in Belgium and the Netherlands from 2014 to 2017. The study protocol was approved by the institutional review board and ethics committee of the Erasmus MC University Medical Center (MEC-2013-300) and by the institutional review boards and ethics committees from each participating site, and all enrolled patients provided written informed consent. Patients were treated with suppositories for 4 weeks and were randomly assigned to either beclomethasone 3mg once daily or tacrolimus 2mg once daily. All study procedures were conducted in accordance to the Declaration of Helsinki. This trial was registered at the Netherlands Trial Register (NL4205, NTR4416). All authors had access to the study data and reviewed and approved the final manuscript.

Patients

Patients aged >=18 years with endoscopically proven active UP, with disease activity up to 20 cm beyond the anal verge. Active disease was defined as either a Mayo endoscopic severity subscore[14] of at least 2, or a histological inflammation grade (Geboes score[15]) of at least 2, regardless of total Mayo score. Additional inclusion criteria were either 5-ASA refractory UP (defined as a failure to at least the use of 5-ASA suppositories of a maximum of 1 gram for at least 21 days) or recurring UP (defined as a relapse within 3 months after stopping adequate local 5-ASA therapy). Concomitant treatment with oral 5-ASA, thiopurines, methotrexate or biologicals was allowed if used at a stable dose for at least 12 weeks prior to enrollment.

Key exclusion criteria were: Signs of bacterial pathogens in a stool sample (i.e. Clostridium difficile, Salmonella species, Shigella species, Yersinia species, Campylobacter jejuni), local IBD therapy with 5-ASA enemas within 14 days prior to randomization, any previous tacrolimus treatment, treatment with topical beclomethasone 12 weeks prior to randomization or any other steroid use 4 weeks prior to randomization. Additionally, other significant medical issues such as poor renal function (eGFR <30 mL/min), poor liver function, leucopenia and thrombopenia were reasons for ineligibility. Finally, pregnant or lactating women were excluded.

Randomization and blinding

Randomization was performed centrally by an independent clinical research bureau. Participating sites were to fax or e-mail a request for randomization, which would then

be provided within 24 hours of the request. Randomization occurred per study site, using a 1 : 1 randomization schedule with various block sizes. To ensure blinding, the investigational drugs were custom made for this trial and were of identical in appearance and weight (Tiofarma BV, Oud-Beijerland, the Netherlands). Patients, treating physicians, endoscopists and investigators remained blinded throughout the study. Tacrolimus serum levels were centrally measured during the study and were thus unavailable to the investigators.

Study procedures

After providing written informed consent, a screening period of up to two weeks prior to randomization started. During this period the index endoscopy had to be performed, confirming the key inclusion criterion of active proctitis as described above. Additionally, baseline laboratory tests and stool cultures were performed to ensure eligibility. Upon eligibility, patients visited the study site for baseline clinical activity measurements and subsequently the study drugs were provided to the patients. Follow-up visits occurred after two and four weeks of treatment. During these visits adverse events were registered, drug accountability was performed and blood samples were acquired. Additionally a second clinical and endoscopic evaluation was scheduled after four weeks of treatment.

Outcome measures

Clinical activity was measured using the Mayo score[14] (see Supplemental data), which consists of 3 clinical variables and 1 endoscopic variable, all rated from 0 to 3. The total score therefore varies between 0 to 12, with a higher score indicating more severe disease. Additionally, histological inflammation was graded using the Geboes score, which ranges from 0 (structural changes only) to 5 (erosions or ulcers)[15]. Grades 0 and 1 are considered remission whereas grades 2 to 5 are considered active disease.

The primary outcome of this study was clinical response after 4 weeks of treatment, defined as a an absolute decrease in Mayo score of ≥3 points, with a relative decrease of ≥30% of the total score and at least ≥1 point decrease in the rectal bleeding subscore or an absolute rectal bleeding subscore of 0 or 1.
Secondary outcomes were combined clinical and endoscopic remission. Clinical remission was defined as a Mayo score ≤2, and endoscopic remission as no visible inflammation (i.e. Mayo sub-score 0). Additional secondary outcomes were endoscopic response, defined as a decrease in Mayo sub-score of ≥1 and/or a decrease in extent of inflammation of ≥5 cm, changes in histological inflammation grade, changes in

C-reactive protein (CRP) and leucocyte counts, adverse events and quality of life using the Dutch version of the inflammatory bowel disease questionnaire (IBDQ)[16].

Statistical analyses

A power analysis was performed using Pearson Chi-squared test for two proportions. Under the assumptions of a 50% response rate for topical steroids and 80% response rate for topical tacrolimus, and with a one-sided alpha of 0.025, >80% power could be achieved with 40 patients in each arm. To account for possible loss to follow-up, it was decided to include an additional 10% of patients, resulting in a total of 88 study patients.

For the statistical analysis the SPSS 24.0 software package was used. Descriptive statistics were used to summarize the data. Medians with the range were calculated for continuous data and percentages were calculated for categorical data.

Apart from missing data, no adjustment for confounders was performed. Categorical data in unrelated groups were compared by the Fisher's exact test, categorical data in related groups were analyzed by McNemar`s test. The Mann-Whitney test was used to compare continuous data. For paired test, the paired sample t-test was utilized. Correlations were assessed using Spearman's rho. For all these results, one or two-sided (as appropriate) P-values <0.05 were considered significant. Analyses were performed according to both intention to treat and per protocol principles. As there were no meaningful differences between these analyses, the per protocol results were reported in this manuscript.

As for missing data, only missing data in the IBDQ was imputed. At baseline, imputation was only performed if up to 3 missing sub-scores were present and no more than 1 sub-score was missing from the "systemic symptoms" or "social functioning" domains, as these domains consist of only 5 questions. In case of a missing sub-score, the lowest possible score (1 point) was imputed. For the IBDQ at week 4, missing values were carried forward from baseline where available, or similarly imputed.

Results

Patient characteristics

Between February 2014 and November 2017, a total of 88 patients were enrolled in this study. However, one patient was subsequently excluded because of protocol

violations (upon monitoring, concomitant use of corticosteroids was discovered). Additionally, 2 patients were excluded because of low Mayo scores at baseline, resulting in 85 patients for per protocol analysis (see Figure 3.1). In total, 43 patients received tacrolimus and 42 received beclomethasone (see Table 3.1). Fifty-seven patients were female (64.7%), median age was 42.3 years (range 18.3-76.4 years) and median disease duration was 7.0 years (range 0.25-47.83 years). Concomitant medication included oral 5-ASA in 39 patients (45.9%), immunomodulators in 16 patients (18.8%) and biologicals in 9 patients (10.6%). The available data are also summarized in Figure 3.1. The study ended after the last follow-up visit from the last patient, in December 2017.

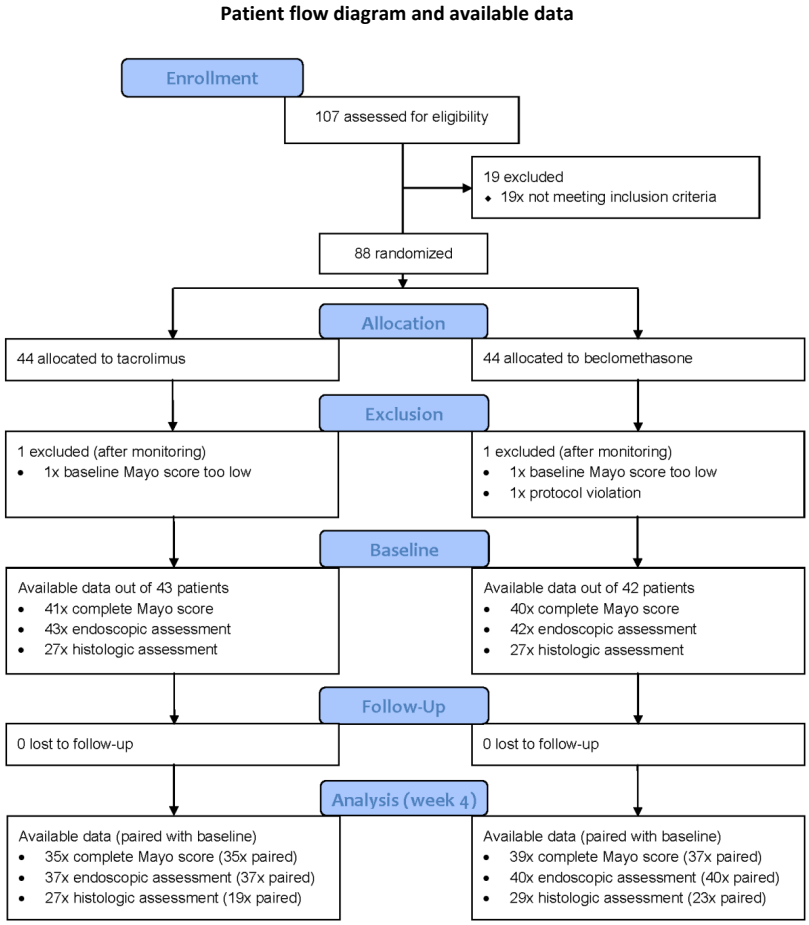

Figure 3.1 CONSORT flowdiagram of screened and included patients, and available study data per timepoint.

Table 3.1 Baseline characteristics.

	Tacrolimus (N = 43)	Beclomethasone (N = 42)
Female (n, %)	27 (62.8%)	28 (66.7%)
Age in years (median, range)	39.6, (18.3 - 75.1)	43.2 (18.6 - 76.4)
Disease duration in years (median, range)	5.8 (0.3 - 36,7)	7.4 (0.3 - 47.8)
Concomitant medication use (n, %)		
Oral 5-ASA	15 (34.9%)	24 (57.1%)
Immunomodulators	10 (23.3%)	6 (14.3%)
Biologicals (anti-TNF n = 8, vedolizumab n = 1)	4 (9.3%)	5 (11.9%)
Smoking status (n, %)		
Current	4 (9.3%)	5 (11.9%)
Former	17 (39.5%)	19 (45.2%)
Never smoked	21 (48.8%)	17 (40.5%)
Total Mayo score (median, range)	7 (3 - 12)	7 (3 - 12)
Mayo endoscopic subscore (n, %)		
0	0 (0%)	0 (0%)
1	4 (9.3%)	2 (4.8%)
2	26 (60.5%)	29 (69.0%)
3	13 (30.2%)	11 (26.2%)
Disease extent in cm (median, range)	10 (2 - 20)	13 (1 - 20)
C-reactive protein (median, range)	2,5 (0.3 - 248,0)	2,0 (0.0 -44.0)
IBDQ (median, range)	146 (91- 211)	145 (87 - 210)

Clinical response

In the tacrolimus group, the median baseline Mayo score was 7 (range 3-12), in the beclomethasone group the median baseline Mayo score was also 7 (range 3-12).

After 4 weeks of treatment, in the tacrolimus group 22 out of 35 patients (62.9%) achieved the primary outcome of clinical response, compared to 22 out of 37 (59.5%) patients in the beclomethasone group, a non-significant difference (P=0.812, Figure 3.2A).

At the end of the study, in the tacrolimus group the median Mayo score decreased to 3 (range 0-12, median change -3.0 points) and in the beclomethasone to 3 (range 0-11, median change -3.5 points, P=0.638).

The secondary outcome of clinical remission was achieved in 16 of 35 patients (45.7%) in the tacrolimus group and 15 of 39 patients (38.5%) in the beclomethasone group, which was not a statistically significant difference (P=0.638, Figure 3.2B).

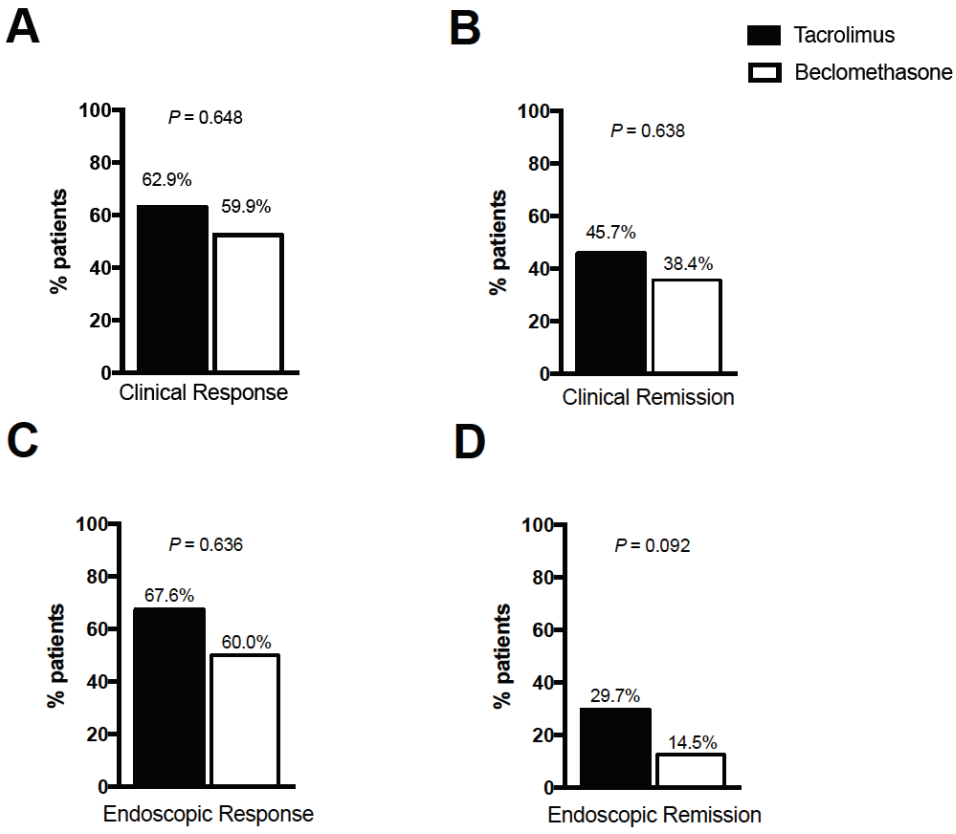

Figure 3.2 Main study results. All panels show tacrolimus as white and beclomethasone as gray. Panel A shows the proportion of patients with a clinical response, panel B shows the proportion of patients with clinical remission. In panel C the proportion of patients with endoscopic response is shown and panel D shows the proportion of patients with endoscopic remission.

Endoscopic response

At baseline, 39 patients (90.7%) in the tacrolimus group and 40 patients (95.3%) in the beclomethasone group had moderate or severe disease activity. The remaining patients had mild endoscopic disease activity, but were included because of severe histological inflammation. Median baseline disease extent was 10cm (2-20) in the tacrolimus group and 12cm (1-20) in the beclomethasone group.

At the end of the study, endoscopic response was achieved in 25 of 37 patients (67.6%) in the tacrolimus group and 24 of 40 (60.0%) patients in the beclomethasone. This difference was not statistically significant (P=0.636, Figure 3.2C).

The difference in endoscopic remission rate was not significantly different (P=0.092), with remission occurring in 11 of 37 patients (29.7%) in the tacrolimus group and in 5 of 40 patients (12.5%) in the beclomethasone group (Figure 3.2D). The change in length of inflamed colon was also not significantly different between both groups (P=0.139).

Histological response

The baseline biopsies showed a median inflammation grade of 3 (range 1-5) in the tacrolimus group and 4 (range 0-5) in the beclomethasone group. At the end of the study, the median inflammation grade decreased to 2 (range 0-5) for both groups. Histological remission was seen in 11 tacrolimus patients (40.7%) and 8 beclomethasone patients (27.6%), which was not a statistically significant difference (P=0.299).

Biochemical parameters

At the start of the study, the median CRP levels in the tacrolimus and beclomethasone groups respectively were 2.5 (range 0.3-248.0) and 2.0 (range 0.0-44). At the end of the study the medians were respectively 2.0 (range 0.3-31.0) and 1.6 (range 0.0-17.0), which was not significantly different (P=0.554).

Median leucocyte counts at baseline in the tacrolimus and beclomethasone groups respectively were 7.2 (range 2.7-13.5) and 6.9 (range 3.0-10.2). After 4 weeks, the medians respectively changed to 7.5 (range 2.8-13.4) and 7.3 (range 3.0-11.7), which was also not significantly different (P=0.476).

Quality of life

At the start of the study, the median IBDQ scores for the tacrolimus and beclomethasone groups were 147 (range 91-211) and 145 (range 87-210) respectively. After treatment, the median increases in IBDQ scores were 18 (range -18-93) and 20.5 (range -13-71), leading to median IBDQ scores of 175 (range 57-214) and 165 (range 80-214), for the tacrolimus and beclomethasone groups respectively, which was not significantly different (P=0.733).

Additionally, changes in the IBDQ were significantly correlated with changes in the total Mayo score (R^2=0.151, P<0.001), endoscopic severity (R^2=0.104, P=0.007) and endoscopic disease extent (R^2=0.164, P<0.001), but not to changes in CRP, leucocyte count or histologic inflammation grade (P=0.766, 0.575 and 0.108 respectively).

Tacrolimus levels and adverse events

Sixty-two tacrolimus levels were available from 37 of tacrolimus treated patients. The mean tacrolimus level was 4.2 ± 3.4µg/L (range 0.0-13.6) at week 2 and 2.7 ± 2.8µg/L (range 0.0-12.8) at week 4. Although tacrolimus levels did not represent trough levels, 46 of the levels (74.2%) were undetectable or subtherapeutic (<5 µg/L). The remainder were within the low therapeutic range (5-20µg/L). There was no correlation between tacrolimus levels and clinical and endoscopic outcome.

Forty-eight adverse events were reported that were judged to be at least possibly related to the study drugs (see Table 3.2 for details). Eighteen adverse events occurred in 14 patients (33.3%) of the beclomethasone group whereas 29 events were seen in 21 patients (48.8%) in the tacrolimus group, which was not significantly different (P=0.188). Within the tacrolimus group, serum tacrolimus levels were not associated with the occurrence of adverse events (P=0.611).

No serious adverse events were reported, nevertheless, one patient discontinued the study due to an adverse event possibly related to the study drug. Specifically, this patient was randomized to tacrolimus and developed a clostridium infection after two weeks.

Table 3.2 Adverse events.

	Tacrolimus	Beclomethasone	Both
Abdominal pain / worsening of symptoms	3	3	6
Arthritis	0	1	1
Peri-anal effects (burning/itching/hemorrhoid/fissure)	9	3	12
Clostridium infection	1	0	1
CMV	1	0	1
Nausea/dizziness/weakness	2	1	3
Skin (flushing, erythema, itchiness)	3	4	7
Flatulence	5	2	7
Headache	2	1	3
Rectal urgency	1	0	1
Night sweats	1	0	1
Palpitations	1	1	2
Upper airway infection	0	2	2
Total	**29**	**18**	**47**

Discussion

In this randomized controlled trial comparing tacrolimus suppositories with beclomethasone suppositories as induction therapy for 5-ASA refractory ulcerative proctitis, no superiority of tacrolimus was shown over beclomethasone. After 4 weeks of treatment, clinical and endoscopic response (62.9% versus 59.9% and 67.6% versus 60%) and clinical and endoscopic remission (45.7% versus 38.5% and 29.7% versus 14.5%) were equal.

Thus, both study drugs managed to induce clinical and endoscopic response in the majority of patients. Furthermore, both treatments resulted in improvements in histological inflammation and quality of life. Adverse event rates were similar in both groups.

To our knowledge, this is the first head to head controlled trial examining the effects of tacrolimus and beclomethasone suppositories in ulcerative colitis patients. The clinical response and remission rates of 60% and 40% respectively are comparable to the rates of other topical corticosteroids[17], though some of these studies examined a combination of UP patients and patients with left-sided disease[18,19].

Topical tacrolimus has been investigated in only few studies. When comparing our study to the randomized controlled trial of Lawrance et al[13], certain differences in the reported outcomes warrant consideration. In their 8-week trial comparing rectal tacrolimus with placebo in patients with therapy refractory UP, they observed a clinical response rate of 73%. Our 4 week study finds a somewhat lower clinical response rate of 62.9% in the tacrolimus group, and finds no statistically significant difference when compared with another active drug. The clinical remission rates are more similar, with Lawrance et al reporting 45% and our study finding 45.7%. However, mucosal healing (defined in their study as an endoscopic Mayo score of 0 or 1) was reported in 73% of their patients, whereas using this criterium it was seen in only 58% of tacrolimus treated patients in our study.

Possible explanations for the differences in clinical response rate and mucosal healing are the differences in baseline characteristics between the patients of these two studies. Specifically, we had a greater proportion of female patients, had more current smokers and patients used more concomitant immunosuppressive and biological drugs. This may reflect more refractory disease in the patients enrolled in our trial, as current guidelines recommend the use of these agents only for refractory UP.

A notable difference between our study and Lawrance et al is a shorter treatment duration (4 weeks compared to 8 weeks).

Additionally, Lawrance et al used a different treatment regimen, consisting of twice-daily rectally applied ointment with a total daily dose of 3mg tacrolimus. In our study we decided to use once daily suppositories containing only 2mg of tacrolimus, based on our previous Phase 1 study[11]. In that study, low but measurable serum levels of tacrolimus were found with the use of once daily 2mg tacrolimus suppositories. Thus in order to prevent systemic exposure to higher tacrolimus levels, the same dose was used in our current study. Concerning serum tacrolimus levels, Lawrance et al also find measurable serum levels, and similar to our study, they find no correlation between serum levels and efficacy or adverse events. Of note, the tacrolimus serum levels in our study do not represent true trough levels, nevertheless the majority of tacrolimus levels were sub-therapeutic. Therefore, the true tacrolimus trough levels would probably be even lower than currently measured. These differences in treatment duration, regimen and patient characteristics may partly explain the differences seen between these studies in the reported in clinical response and mucosal healing rates.

No serious adverse events were reported during the study period, and only a single adverse event, possibly related to the study drugs, caused patients to discontinue the study. Nevertheless, mild adverse events, particularly peri-anal itching and burning, were frequently reported, more often in patients treated with tacrolimus.

Systemically applied calcineurin inhibitors are already approved for use in steroid refractory acute severe ulcerative colitis[7]. The optimal position of topical tacrolimus within the step-up scheme for treatment of UP is currently unclear. Topical 5-ASA is the first line therapy for UP patients because of the robustly proven efficacy and side effect profile. Given the results of our study and of previous studies, using either topical tacrolimus or topical corticosteroids as the second step in UP therapy both seem safe and viable options, and both therapies should be considered prior to step-up to immunomodulators or biologicals.

Despite the randomized controlled and triple blinded design, there are limitations to this study. Firstly, patients were mostly enrolled in tertiary centers. This may have resulted in the inclusion of patients with more severe disease than seen in general clinical practice.

Secondly, the inclusion criteria of our study were primarily based on the presence of endoscopic disease activity. Though this was intended to ensure objective disease activity at the start of the study, some of the included patients had surprisingly low Mayo scores at inclusion. These low baseline scores may have reduced the amount of patients who could achieve the predefined 3 point decrease in the Mayo score to achieve the primary outcome of clinical response, thus resulting in a study with less power than initially designed.

Thirdly, this study only examined an induction treatment period of 4 weeks, thus the value of a longer induction period or (intermittent) maintenance therapy remains unclear. Initially, a study period of 8 weeks was considered, but due to concerns regarding side effects related to long-term rectal corticosteroid therapy a 4 week study period was chosen.

In summary, among 5-ASA refractory ulcerative proctitis patients, the use 4 weeks of tacrolimus suppositories was not superior to treatment with beclomethasone suppositories. Furthermore, no significant differences between tacrolimus and beclomethasone were seen regarding the secondary outcomes and both treatments appear to be safe. Therefore, both tacrolimus suppositories and beclomethasone suppositories appear to be viable treatment options for 5-ASA refractory disease. Topical treatment with tacrolimus should be considered prior to step-up to thiopurines or biologicals in 5-ASA refractory ulcerative proctitis.

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Supplemental data

Mayo score, exactly as described by Schroeder KW, Tremaine WJ and Ilstrup DM (N Engl J Med. 1987 Dec 24;317(26):1625-9.).

Stool frequency*
0 = Normal no. of stools fort his patient
1 = 1-2 stools more than normal
2 = 3-4 stools more than normal
3 = 5 or more stools than normal

Rectal bleeding¥
0 = No blood seen
1 = Streaks of blood with stool less than half the time
2 = Obvious blood with stool most of the time
3 = Blood alone passed

Findings of flexible proctosigmoidoscopy
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's global assessment ‡
0 = Normal
1 = Mild disease
2 = Moderate disease
3 = Severe disease

* Each patient served as his or her own control to establish the degree of abnormality of the stool frequency; ¥ The daily bleeding score represented the most severe bleeding of the day; ‡ The physician's global assessment acknowledged the three other criteria, the patient's daily record of abdominal discomfort and general sense of well-being, and other observations, such as physical findings and the patient's performance status.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	4
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	6
	2b	Specific objectives or hypotheses	6-7
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	n.a.
Participants	4a	Eligibility criteria for participants	7-8
	4b	Settings and locations where the data were collected	7
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9-10
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	10-11
	6b	Any changes to trial outcomes after the trial commenced, with reasons	n.a.
Sample size	7a	How sample size was determined	11
	7b	When applicable, explanation of any interim analyses and stopping guidelines	n.a.
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	9
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	9
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	9
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	9
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	9
	11b	If relevant, description of the similarity of interventions	9
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	11-12
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	11-12

Section/Topic	Item No	Checklist item	Reported on page No
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	12, Fig 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	Fig 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	12
	14b	Why the trial ended or was stopped	12
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	13-16, Table 2
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	13-16
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	n.a.
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	n.a.
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	15-16
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	17-18
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	16-17
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	16-18
Other information			
Registration	23	Registration number and name of trial registry	5, 8
Protocol	24	Where the full trial protocol can be accessed, if available	n.a.
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	2

Chapter 4

Low dose naltrexone for induction of remission in inflammatory bowel disease patients

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Abstract

Background

Around 30% of patients with inflammatory bowel disease (IBD) are refractory to current IBD drugs or relapse over time. Novel treatments are called for, and low dose Naltrexone (LDN) may provide a safe, easily accessible alternative treatment option for these patients. We investigated the potential of LDN to induce clinical response in therapy refractory IBD patients, and investigated its direct effects on epithelial barrier function.

Methods

Patients not in remission and not responding to conventional therapy were offered to initiate LDN as a concomitant treatment. In total 47 IBD patients prescribed LDN were followed prospectively for 12 weeks. Where available, endoscopic remission data, serum and biopsies were collected. Further the effect of Naltrexone on wound healing (scratch assay), cytokine production and endoplasmic reticulum (ER) stress (GRP78 and CHOP western blot analysis, immunohistochemistry) were investigated in HCT116 and CACO2 intestinal epithelial cells, human IBD intestinal organoids and patient samples.

Results

Low dose Naltrexone induced clinical improvement in 74.5%, and remission in 25.5% of patients. Naltrexone improved wound healing and reduced ER stress induced by Tunicamycin, lipopolysaccharide or bacteria in epithelial barriers. Inflamed mucosa from IBD patients showed high ER stress levels, which was reduced in patients treated with LDN. Cytokine levels in neither epithelial cells nor serum from IBD patients were affected.

Conclusions

Naltrexone directly improves epithelial barrier function by improving wound healing and reducing mucosal ER stress levels. Low dose Naltrexone treatment is effective and safe, and could be considered for the treatment of therapy refractory IBD patients.

Background

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder, which includes Crohn's disease (CD) and ulcerative colitis (UC). The aim of therapy is to induce sustained remission, a state of long lasting quiescent disease. Several drugs exist to induce and maintain remission, and these drugs are usually prescribed in a step-up fashion. Nevertheless, even when using this step-up strategy, a subset of patients will fail to reach or maintain remission even with the most potent therapies, often due to drug intolerance or loss of efficacy. For instance, yearly loss of efficacy rates are 13-24% in patients treated with anti-tumor necrosis factor α (anti-TNF-alpha) agents[1,2]. In general, such a state of therapy refractoriness occurs in 35–60% of all IBD patients and severely limits treatment options, often resulting in surgery or corticosteroid dependency[3,4]. Thus, for this subset of patients, alternative treatments remain of continued interest.

The etiology of IBD is complex, with genetic predisposition, an altered microbiome, environmental factors and a weakened epithelial barrier function triggering a chronic mucosal immune response. Targeting these different causes with medication is the current challenge in IBD treatment. Previous research suggests that the endogenous opioid system also plays a role in gut immunity[5,6]. For instance, in IBD patients, the μ-opioid receptor (MOR) is overexpressed in mucosal T-lymphocytes and monocytes, and ex vivo stimulation of MOR with the agonist DALDA reduced TNF-alpha in mucosal biopsies from IBD patients[7]. In addition, DALDA also showed anti-inflammatory responses in a mouse model of colitis through inhibition of T cell proliferation and cytokine (including TNF-alpha) production[8]. Another opioid known to modulate MOR responses is Naltrexone. While being a MOR antagonist, which blocks endogenous opioid effects when used at high concentrations[9], administration of low dose Naltrexone (LDN) is postulated to result in upregulation of endogenous encephalin and endorphin levels and to have a positive modulatory effect on the MOR[10,11]. Thus, the use of LDN in clinical settings is gaining interest, with Crohn's disease, multiple sclerosis and fibromyalgia described as potential targets for treatment with LDN[12–14]. In both mouse and rat models of IBD, LDN alleviated inflammation, in part by reducing pro-inflammatory cytokine production[15,16]. Interestingly, we and others have shown that endoplasmic reticulum (ER) stress in intestinal Paneth cells is one of the contributing factors in IBD[17–19] and it was recently reported that Naltrexone attenuates inflammation in a mouse liver injury model by reducing ER stress[20,21].

Pilot studies in patients with CD showed a positive effect of LDN therapy, with 15 of 17 patients showing a clinical response[22]. A subsequent randomized, placebo-controlled, double blind study in 34 patients found a response rate of 88% in the LDN

group versus 40% in the placebo group after 12 weeks of therapy[23]. In addition LDN was also shown to be safe in pediatric IBD patients, and resulted in significantly reduced PCDAI scores, with 25% of patients achieving remission and 67% showing improvement of disease[24]. The above results suggest that LDN is an effective therapy for CD patients, although the exact mechanism remains unclear. Based on these promising results, patients started to request LDN therapy due to its favorable side-effect profile, and at the Erasmus MC we decided to start prescribing LDN to therapy refractory IBD patients with active disease. The aims of this study were to assess the clinical effect of LDN and to investigate whether LDN has a direct modulatory effect on intestinal epithelial barrier function.

Methods

Clinical cohort

A prospective cohort of patients with therapy refractory IBD (CD or UC) that started LDN therapy was formed. The decision to start LDN therapy was made by the treating physician, after fully informing the patient of the possible benefits and drawbacks. All patients were prescribed 4.5mg Naltrexone once daily. Patients were instructed to administer one dose of LDN before bedtime.

Upon initiation of LDN therapy, patients were followed according to usual care at the outpatient clinic, with contact (in person or via telephone) after 4, 8 and 12 weeks. During these visits self-assessed disease activity and adverse events were recorded. Patients were offered endoscopic evaluation and assessment of laboratory values after 12 weeks of treatment or at time of discontinuation of LDN therapy, whichever occurred earlier. This study was approved by the Ethical Committee of the Erasmus MC (MEC-2014-656).

Clinical data collection

During the follow-up, demographic data (e.g. age, gender) and IBD related data (e.g. year of diagnosis, concomitant and previous IBD related therapies, Montreal phenotype classification) were recorded. Additionally, where available, data on diagnostic tests, particularly endoscopic evaluations, performed prior to the start of LDN therapy (with a 1 week window) and during the follow-up period were recorded. All patients that completed at least 1 assessment of disease activity were included in the cohort.

Clinical outcome measures

Clinical outcomes were based on patient self-assessments and outpatient assessments, where available. Patients were considered non-responders if no clinical improvement occurred in the first 4 weeks of LDN therapy. Patients were considered to have clinical response if self-assessed disease activity decreased within the first 4 weeks of LDN therapy, and lasted for at least 4 weeks in total. Of secondary interest were the rates of adverse events during LDN therapy. Endoscopy results were scored based on the most severe area of inflammation. Endoscopic findings in all IBD patients were scored on a scale from 0 to 3, representing no inflammation to severe inflammation respectively.

Cell lines

Colorectal cancer cell lines HCT116 and CACO-2 were cultured in Dulbecco's Modified Eagles Medium (DMEM, Lonza, Basel, Switzerland) supplemented with 100U/mL penicillin, 100mg/mL streptomycin (Life technologies, Bleiswijk, NL) and 10% Fecal Calf Serum (FCS, Sigma-Aldrich, St. Louis, USA). Cells were maintained at 37°C in a 5% CO_2 humidified setting.

Organoid culture

Non-inflamed intestinal biopsies were collected from two IBD patients undergoing routine endoscopy for their disease. Organoids were prepared as described[25,26], see Additional file 4.1 for details.

Cell viability assay

Cell viability was assessed using MTT assays as described[27], see Additional file 4.1: Methods. Each experiment was performed twice in triplicate.

Wound healing assay

Wound healing assays were performed as described[28], see Additional file 4.1. The concentration of Naltrexone used was based on in vivo dosages (4.5mg per ± 60 kg bodyweight). Experiments were performed thrice in duplicate, with two measure-sites per scratch.

Western blotting

Western blotting was performed as described[29], with modifications (see Additional file 4.1). HCT116 and CACO-2 cells were treated with Tunicamycin (2µM), lipopolysaccharide (10µg/mL LPS) or E. coli (paraffin-fixed DH5α, 6.25e5/mL) in the presence or absence of 1µg/mL Naltrexone. Organoids were treated with LPS in the presence or absence of 1µg/mL Naltrexone. Experiments were performed at least twice.

Immunohistochemistry

FFPE tissue sections were immunohistologically stained for GRP78, as described[17,30], see Additional file 4.1. Antigen retrieval was performed by boiling the slides in 600mL of 10mM sodium citrate buffer, pH 6.0 for 15 min. Slides were blocked by incubating in 10% goat serum in PBS and incubated with GRP78 antibody (BiP, Cell Signaling Technology, Danvers, MA) diluted in blocking buffer (1:100) overnight at 4°C. Rabbit envision (DAKO, Heverlee, Belgium) was used as secondary antibody.

Reverse transcriptase polymerase chain reaction (rt-PCR)

We used rt-PCR to determine MOR expression on the IEC cell lines, using Ribosomal protein primers were used as control[26], see Additional file 4.1.

Enzyme linked immunosorbent assay (ELISA)

Cells were plated at 0.2 × 106 per well in 24 wells plates. Upon attachment to the plate, cells were treated as described in the text and supernatant was harvested after 24h. Experiments were performed twice, in duplicate. Cytokine levels in supernatants from IECs and patient sera were determined by ELISA (Ready-SET-Go!® eBioscience, San Diego, CA) as per manufacturer's instructions. All samples were tested in duplicate in the ELISAs.

Statistical analysis

Continuous variables were reported as medians with interquartile range (IQR). Comparisons in continuous variables were performed with the Mann-Whitney U test. For comparisons of categorical variables, Fisher's exact test was used. For in vitro and ex vivo experiments, normality of distribution was assessed with D'agostino and Pearson Omnibus normality test. When passing normality test or when there were insufficient numbers to calculate normality, parametric testing was performed,

otherwise, non-parametric tests were employed. Student T-tests were performed for comparisons of two groups. For comparisons of more than two groups, ANOVA with post hoc testing (Tukey's multiple comparison test) was performed. For all tests, one or two-sided (as appropriate) p-values <0.05 were considered statistically significant, graphs show mean ± SEM. Analyses were performed using Graphpad Prism 5.0.

Results

Patient characteristics

From July 2010 till August 2014, 47 patients were treated with LDN, of which 19 (40.4%) were male and 28 were female. Median treatment and follow-up duration after start of LDN was 3 months (IQR 3-5 months). Of the 47 patients, 28 (59.6%) were diagnosed with CD and 19 with UC. Three patients had previously undergone surgery (2 ileocecal resections and 1 subtotal colectomy, all in CD patients). The full baseline patient characteristics are described in Table 4.1.

Table 4.1 Baseline patient characteristics.

General characteristics			
Diagnosis, N (%)	UC, 19 (40%)	CD, 28 (60%)	Combined, 47
Gender (M/F)	10/9	9/19	19/28
Median age at diagnosis (years, IQR)	31 (27-44.5)	23 (16.8-32.5)	27 (18-39.5)
Median age at start of LDN (years, IQR)	42 (33.5-52)	25.5 (25.5-53.5)	40 (27.5-52.5)
Median disease duration at start of LDN (years, IQR)	6.9 (3.2-12.4)	7.8 (3.8-16.5)	7.0 (3.8-13.4)
CRP mg/L, median (IQR)	7 (2-27)	6 (2-7)	6 (2-9)
Endoscopic score (median, IQR)	2.0 (1.0-2.0)	2.0 (2.0-2.0)	2.0 (1.25-2.0)
Disease characteristics			
Disease extent or phenotype [Montreal classification, % (N)]	E1 11% (2) E2 63% (12) E3 26% (5)	L1 7% (2) L2 32% (9) L3 61% (17)	

CD Crohn's disease, CRP C-reactive protein, IQR inter-quartile range, LDN low dose Naltrexone, UC ulcerative colitis.

All participants were either steroid dependent or steroid refractory and had previously been treated with at least one other drug, and all patients showed clinical signs of disease activity at initiation of LDN therapy. Notably, 41 patients (87.2%) had previously received at least one anti-TNF-alpha agent, and 19 (40.4%) had been treated with two anti-TNF-alpha agents. The 6 patients not exposed to anti-TNF-alpha had refused anti-TNF-alpha therapy due to fear of possible side effects. The full details on the previous and concomitant treatments at start of LDN therapy are described in Table 4.2. Seven

patients (14.9%) reported adverse events due to LDN, including vivid dreams (N=4), drowsiness (N=2) and headache (N=1). Two patients discontinued LDN therapy after 2 weeks, due to drowsiness. Vivid dream complaints improved when LDN was administered in the morning instead of at bedtime.

Table 4.2 Medical therapies used prior to start of LDN and concomitantly with LDN.

	UC, 19	CD, 28	Combined, 47
Therapies prior to LDN, N (%)			
5-ASA	17 (89%)	14 (50%)	31 (66%)
Steroids	19 (100%	28 (100%)	47 (100%)
Immunosuppresives	18 (95%)	27 (96%)	45 (96%)
Anti-TNF	16 (84%)	25 (89%)	41 (87%)
Other	5 (26%)	2 (7%)	8 (15%)
Concomitant therapies at start of LDN, N (%)			
5-ASA	7 (37%)	3 (11%)	10 (21%)
Steroids	6 (32%)	18 (64%)	24 (51%)
Immunosuppresives	8 (42%)	9 (32%)	17 (36%)
Anti-TNF	5 (26%)	3 (11%)	8 (17%)
Other	1 (5%)	2 (7%)	3 (6%)
None	4 (21%)	7 (25%)	11 (23%)

Steroids refer to any form of corticosteroids. Immunosuppressives refer to thiopurines or methotrexate. Other refers to tacrolimus, cyclosporine, thioguanine or blinded trial drugs. Anti-TNF anti-tumor necrosis factor, CD Crohn's disease, LDN low dose Naltrexone, UC ulcerative colitis.

LDN therapy shows clinical and endoscopic efficacy in IBD patients

Of the 47 patients, 35 (74.5%) achieved a clinical response. Of those 35 patients, 12 patients had a response of at least 3 months (25.5% of total cohort, 8 CD, 4 UC), whereas a short-lived (between 4 and 12 weeks) improvement was seen in the remaining 23 patients (48.9% of total cohort, 13 CD, 10 UC). There was no statistically significant difference between CD and UC patients in the number of patients that achieved either response or remission (P=1.000 and P=0.515 respectively).

The median endoscopic score at baseline amongst all patients was 2 (IQR 1.25-2.0). In 12 patients, consecutive endoscopies were performed both at baseline and at 12 weeks or at time of relapse, whichever occurred earlier. These consecutive endoscopies were performed in 6 patients with response (3 CD, 3 UC) and 6 patients with remission (1 CD, 5 UC). Between these two groups, no significant difference was observed in baseline endoscopic score (median 1.67, range 1-3 versus 1.83, range 0-3 for response and remission respectively P=0.676). However, patients achieving clinical remission had a significantly greater improvement in endoscopic score compared to patients not reaching clinical response (median change −1.5, range −2 to 0 versus 1.0, range 0-2,

P=0.005, Figure 4.1). Complete endoscopic remission upon treatment with LDN was seen in 5 out of 6 patients with clinical remission.

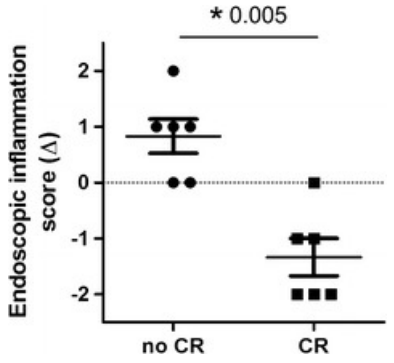

Figure 4.1 Changes in individual endoscopic inflammation scores values. The change in endoscopic score value for patients in clinical remission after week 12 and patients not in clinical remission after week 12 are displayed. Each dot represents an individual. The horizontal line represents the group median. The difference in group medians is statistically significant (−1.5 versus 1.0, for clinical remission and not in clinical remission respectively, p = 0.005).

Naltrexone improves wound healing in intestinal epithelial cell layers

Having shown clinical effect of Naltrexone in patients, we next sought to establish whether Naltrexone has a direct effect on epithelial cell function. After testing for the presence of MOR (Figure 4.2a), we investigated the effect of Naltrexone on wound healing in layers of HCT116 and $CACO_2$ colonic epithelial cell lines. Figure 4.2b shows that scratch wounds inflicted in HCT116 cell cultures are healed significantly faster when cells are treated with Naltrexone as compared to vehicle control (P=0.0001 at t=24; P=0.0001 at t=48). In $CACO_2$ cells, which migrate much faster than HC116, all wounds were healed at t=48 and the effect of Naltrexone on wound size was less clear (t=24h, P=0.085, Figure 4.2b, right panel). Possibly, the lower MOR expression levels observed in $CACO_2$ cells accounts for the lesser effect of Naltrexone in this cell line. However, when comparing the migrated distance of cells, it was evident that Naltrexone stimulated epithelial cell migration, in both HCT116 (361 ± 24 vs. 656 ± 52 pixels, P=0.085) and $CACO_2$ (465 ± 26 vs. 310 ± 50 pixels, P=0.0083, Figure 4.2c). This effect of Naltrexone on wound healing was not due to an increased cellular proliferation, as total viable cell numbers were not affected by Naltrexone up to a concentration of 100µg/mL (Additional file 4.2: Figure S4.1A-C).

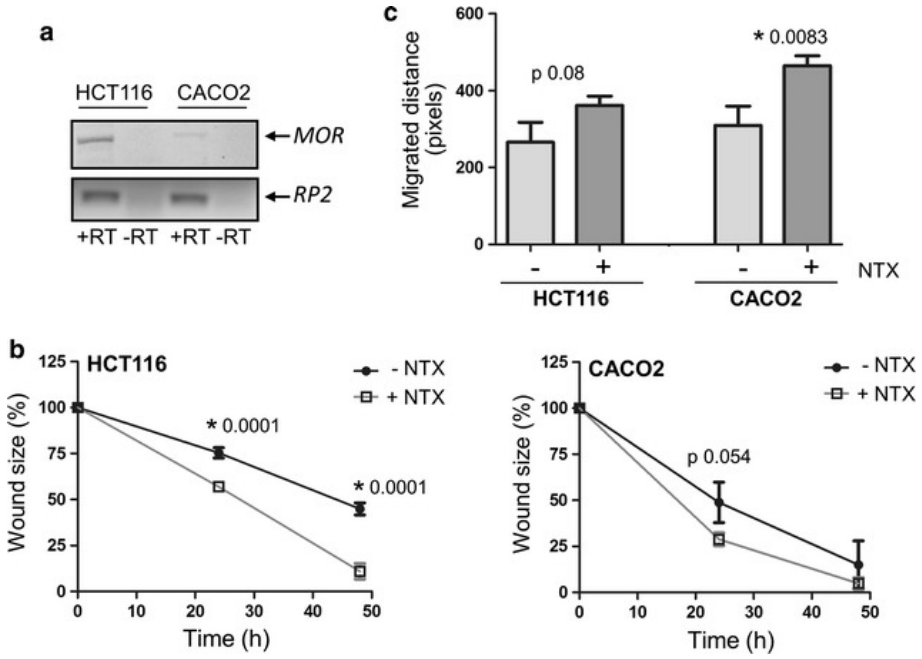

Figure 4.2 Naltrexone improves epithelial wound healing. a Expression of the μ-opioid receptor (MOR) was tested by rt-PCR, using -RT (i.e. RNA) controls. Ribosomal protein 2 (RP2) was used as cDNA quality control. b HCT116 (left panel) and CACO2 (right panel) cultures were scratched in the presence or absence of 1 μM Naltrexone (NTX), and wounds were photographed at t = 0, 24 and 48. Mean percentage wound size of three independent experiments is shown. c Migration of wound edges at t = 24 in pixels presented as mean of three experiments.

Naltrexone does not affect interleukin 8 (IL-8) cytokine levels in epithelial cells and patient sera

In vivo, epithelial cells produce an array of cytokines in response to inflammatory stimuli, which in turn can attract immune cells and perpetuate inflammation in IBD patients. We therefore investigated whether cytokine production by epithelial cells is directly affected by Naltrexone. Cells were stimulated with bacteria in the absence or presence of Naltrexone, and supernatants were tested for the presence of the pro-inflammatory cytokines IL-6, IL-8 and TNF-alpha after 24h. As shown in Figure 4.3, treatment of cells with bacteria significantly increased IL-8 production in both HCT116 and CACO2 cells (44 ± 4 to 92 ± 14 pg/mL in HCT116, P=0.0001, Figure 4.3a and 17 ± 1 to 25 ± 3 pg/mL in CACO2, P=0.0001, Figure 4.3b). However, neither basal levels nor

bacteria-stimulated levels of IL-8 were significantly affected by Naltrexone treatment. IL-6 and TNF-alpha levels were undetectable (not shown).

Next we tested whether IL-8 or TNF-alpha systemic levels in patients were modulated by Naltrexone treatment in vivo. Paired serum samples (before and after initiation of treatment) were available of 7 patients, 3 responders and 4 non-responders. IL-8 was detected in 6 patients (Figure 4.3c), whereas TNF-alpha could be measured in serum from 5 out of 7 patients (Figure 4.3d). No consistent up or down modulation of either IL-8 or TNF-alpha was observed, nor were there significant differences in these cytokine levels between responders and non-responders to Naltrexone (Figure 4.3e, 4.3f). Together, these data suggest that Naltrexone does not positively impact on inflammation through modulation of intestinal epithelial cell cytokine production.

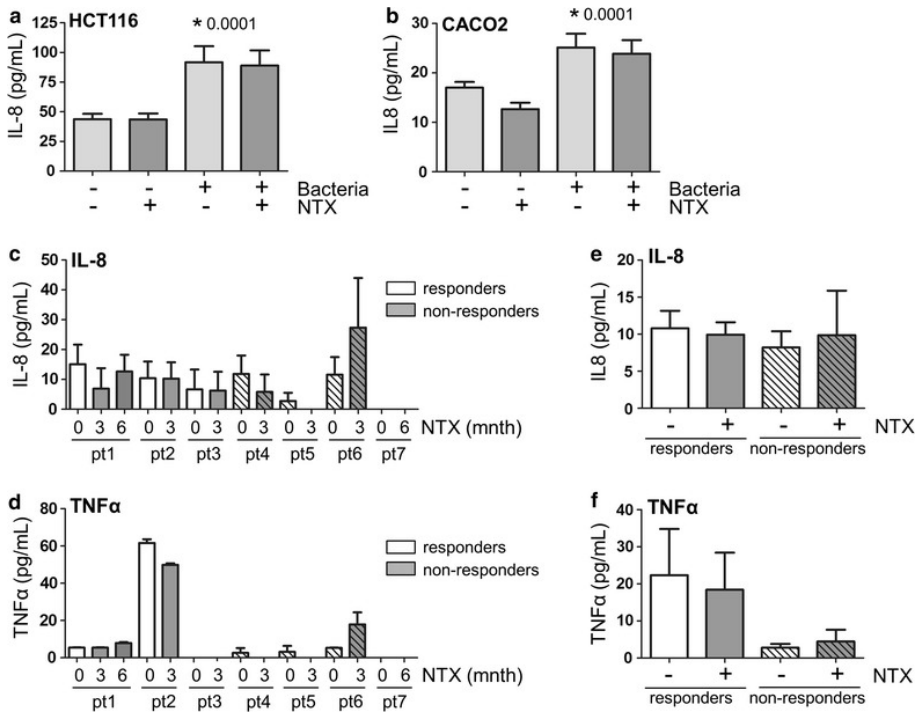

Figure 4.3 Naltrexone does not affect IL-8 levels in epithelial cell lines and patient sera. Stimulation of HCT116 (a) and CACO2 (b) cell layers for 24 h with bacteria results in significantly increased IL-8 levels in culture supernatants as determined by ELISA. Co-treatment with 1 µg/mL Naltrexone does not affect basal levels or bacteria-induced levels of IL-8 production in these cell lines. c–f Serum from patients was taken before low dose Naltrexone (NTX) treatment, and 3 or 6 months into treatment. IL-8 was detectable in 6 of 7 patients c by ELISA, whereas TNF-alpha was detectable in 5 patients (d). There was no significant difference in the mean IL-8 (e) or TNF-alpha (f) levels between responders and non-responders to low dose Naltrexone.

ER stress is intestinal epithelium is reduced by Naltrexone

As ER stress in the mucosa has been associated with the development of IBD, and Naltrexone was previously shown to reduce ER stress-induced inflammation in a model of liver damage, we next investigated whether Naltrexone has a direct effect on ER stress in intestinal epithelium. ER stress was chemically induced in intestinal epithelial cell lines by Tunicamycin (Figure 4.4a and Additional file 4.3: Figure S4.2), as demonstrated by a strong upregulation of the ER stress marker GRP78. Interestingly, Naltrexone was able to reduce these levels in both cell lines. Chemical stimulation of cells with Tunicamycin causes inhibition of the UDP-N-acetylglucosamine-dolichol phosphate N-acetylglucosamine-1-phosphate transferase (GPT) and subsequent accumulation of unfolded glycoproteins in the ER; a non-physiological process likely to result in much higher ER stress levels than are probable in vivo. To investigate ER stress in a more physiologically relevant setting, we incubated intestinal epithelial cells with bacteria (representative examples shown in Figure 4.4b and Additional file 4.3: Figure S4.2A). A significant upregulation of GRP78 expression was observed in HCT116 cells treated with E. coli (0.065 ± 0.007 to 0.097 ± 0.01, P=0.0025, Figure 4.4b), which again was significantly reduced by co-treatment of cells with Naltrexone (0.076 ± 0.005, P=0.0025). In $CACO_2$ cells, Naltrexone diminished bacteria-induced GRP78 expression in three out of three experiments, although this did not reach statistical significance as bacteria-induced ER stress was low in these cells (Additional file 4.3: Figure S4.2A). In order to further confirm ER stress pathway activation with a different physiological stimulus, we also induced ER stress in HCT116 cells with lipopolysaccharide and investigated expression levels of CHOP, a downstream target of the ER stress pathway. Figure 4c shows that CHOP levels induced by LPS were reduced by co-treatment with Naltrexone (1.315 ± 0.592 to 0.801 ± 0.710, P=0.027). Again, the effect was less clear for $CACO_2$ cells (Additional file 4.3: Figure S4.2C).

While cell lines are an easy and common model system to study epithelial cell function, such cell lines may show different cellular effects due to transforming mutations. We therefore generated organoids from colonic biopsies from two IBD patients, representing IBD epithelial tissue. Stimulation of these organoids with LPS again induced GRP78 expression (4 out of 4 experiments), and ER stress levels were reduced by co-treatment of organoids with Naltrexone (Figure 4.4d, 1.734 ± 0.473 to 1.017 ± 0.698, P=0.046). Together, these data imply that ER stress in intestinal epithelial cells is alleviated by Naltrexone.

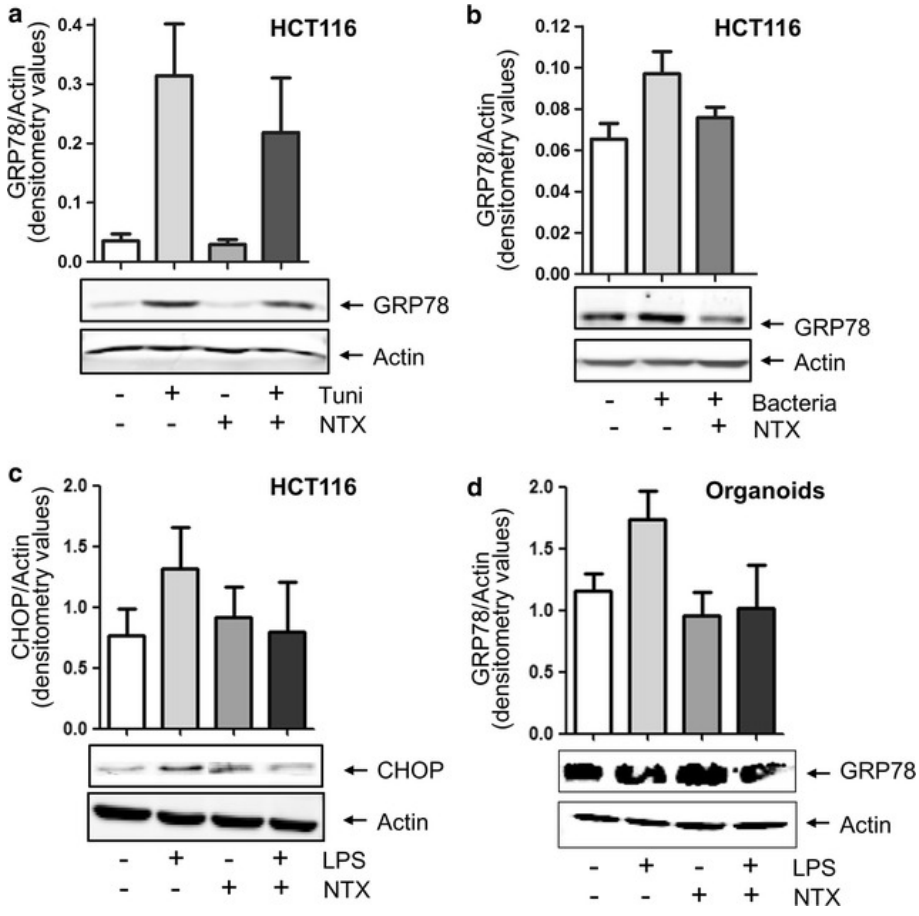

Figure 4.4 ER stress in epithelial cell lines is decreased by Naltrexone. a ER stress was induced in HCT116 cells by treatment with 2 μM Tunicamycin (Tuni), resulting in an upregulation of GRP78 expression levels as detected by Western Blot analysis. Co-treatment of cells with 1 μg/mL Naltrexone (NTX) reduces the amount of Tunicamycin-induced GRP78 expression. Upper graph: mean densitometry values of two independent experiments, GRP78 expression is corrected for Actin, to control for equal loading. Representative example is shown in the bottom panels. b Treatment of HCT116 cells with bacteria results in a significant upregulation of GRP78 expression as detected by Western blot analysis, which is reduced by co-treatment cells by treatment of cells with 1 μg/mL Naltrexone. Mean densitometry values of four independent experiments is shown. c Treatment of HCT116 cells with LPS results in a significant upregulation of CHOP expression as detected by Western blot analysis, which is reduced by co-treatment cells by treatment of cells with 1 μg/mL Naltrexone. Mean densitometry values of three independent experiments is shown. d Treatment of organoids with LPS results in a significant upregulation of GRP78 expression as detected by Western blot analysis, which is reduced by co-treatment cells by treatment of cells with 1 μg/mL Naltrexone. Mean densitometry values are shown of experiments performed on organoids derived from two individual donors, with two independent experiments each.

Next, we investigated intestinal ER stress in patients treated with LDN. Intestinal tissue biopsies were available in 13 patients prior to treatment and in 5 patients 3 months into treatment, with 3 paired samples. Sections were stained for GRP78 (for specificity of the staining, see Additional file 4.4: Figure S4.3). High levels of ER stress were observed in both the inflamed intestinal lamina propria and crypts from IBD patients (Figure 4.5a). GRP78 levels decreased upon NTX treatment, most noticeably in the lamina propria, (1.14 ± 0.5 vs. 0.8 ± 0.5 for lamina propria and 0.9 ± 0.4 vs 0.7 ± 0.6 for crypts) although statistical significance was not reached because of low patient numbers (Figure 4.4b). However, in the 3 paired samples available, NTX treatment reduced interstitial ER stress (Figure 4.4c, and examples in Figure 4.4d). In toto, these data suggest that Naltrexone has a direct effect on ER stress as measured by GRP78 expression in intestinal mucosa.

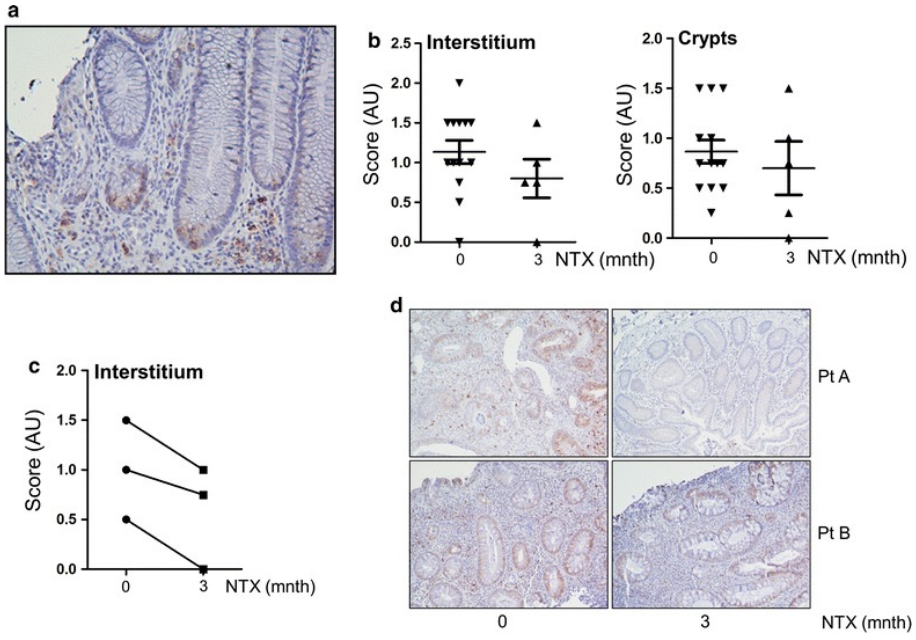

Figure 4.5 High ER stress in mucosa from IBD patients is reduced by low dose Naltrexone treatment. a Example of GRP78, showing high ER stress marker expression in crypts as well as lamina propria. b GRP78 intensity was scored in lamina propria and crypts from biopsies taken from 13 patients before start of low dose Naltrexone and 5 patients 3 months into treatment. Lower levels of GRP78 expression were observed in lamina propria, although this did not reach statistical significance. c Paired biopsies were available from three patients. All three showed reduction of GRP78 expression in the lamina propria upon treatment with low dose Naltrexone. d Two paired samples are shown. Patient A was a non-responder, Patient B did show clinical response to low dose Naltrexone.

Discussion

In this study, therapy refractory IBD patients receiving LDN showed clinical improvement in 74.5% of all patients and long-lasting clinical remission of in 25.5%. Furthermore, most patients achieving clinical remission also showed endoscopic improvement. The response and remission rates in this study appear slightly lower than the rates found in previously published studies (response rates of 88–89% and remission rates of 30-67%[22,23]). These differences might be explained by differences in patient population, as the patients in our cohort had more severe disease, as reflected by the differences in previous drug exposure. Furthermore, the sample size of the previous studies was small, with only 17 and 18 patients receiving Naltrexone in the pilot study and the placebo controlled study, respectively. No serious adverse events were reported in the current study. Interestingly, we also found no elevated liver enzymes in our cohort, whereas previous studies found such abnormalities in 1.8-11.1% of patients treated with Naltrexone[22,23]. Thus, our data suggest that LDN is safe and effective in the treatment of conventional therapy-refractory IBD patients.

While the potential benefit of Naltrexone treatment for IBD is becoming clear, the underlying mechanisms and the general role of the opioid system in IBD have so far received very little attention. An increased expression of MOR in mucosal immune cells has been shown, and one possible function of this upregulation may be compensatory pain management. Pro-inflammatory Th1 and Th17 cells produce enhanced levels of endogenous opioids during colitis in mice[31], which suppress pain signals during chronic mucosal inflammation[32]. As such, it is conceivable that part of the remission in LDN treated patients is a result of a general improvement of well-being. Interestingly, antagonists of the nociceptor receptor (involved in pain sensation) also reduced intestinal pro-inflammatory cytokine profiles and ameliorated DSS colitis in mice, suggesting that blocking pain sensors has a direct immune-modulatory effect.[33] Intriguingly, it has recently been shown that the opioid inactive (+)-isomers of Naltrexone inhibit lipopolysaccharide-induced Toll like Receptor 4 (TLR4) signaling, a bacterial-induced inflammatory pathway contributing to IBD[34,35]. It is as yet unclear whether the Naltrexone preparations currently used in patients (and as bought for in vitro experiments) contain this opioid inactive isoform, but it is at least theoretically possible that some of the beneficial effects observed in the current study are not regulated by MOR, but rather by inhibition of TLR signaling. Furthermore, in addition to MOR, Naltrexone also has weak affinity for the κ and δ opioid receptors, and it is conceivable that some of the observed effects occur through these receptors.

The limited studies performed so far on the mechanistic effect of Naltrexone have mainly focused on immune cells. However, our study suggests that Naltrexone can also have direct beneficial consequences on epithelial barrier cells, by stimulating wound healing. These data are in accordance with the improved in vivo wound healing observed upon Naltrexone treatment in both IBD patients and diabetic mice[36]. However, while the effect of Naltrexone on wound healing in skin was shown to be a result of increased fibroblast proliferation[37], our in vitro model suggests that wound healing of intestinal epithelial barriers is modulated by improved migration rather than proliferation.

Other studies investigating the potential mechanism of LDN on inflammation have focused on immune cell cytokine production. Elevated TNF-alpha, IL-6 and IL-12 levels have been reported to be reduced by Naltrexone in chemically induced mouse colitis models[15,16]. In contrast, others have found that LDN enhances dendritic cell maturation and stimulates their TNF-alpha and IL-12 production, whereas in the current study, no effect of Naltrexone on either epithelial induced IL-8 production or IL-8 and TNF-alpha serum levels in IBD patients was observed. However, it should be noted that not all cytokines could be detected in our system, and it is possible that other cytokines, which were not studied here, are affected.

We and others have previously shown that patients carrying gene variants associated with development of IBD demonstrate increased mucosal ER stress and bacterial persistence, suggesting that intestinal ER stress contributes to IBD pathology[19,38-40]. The cell type that appeared most affected, even in non-inflamed mucosa, were the Paneth cells, specialized anti-microbial peptide producing cells[17]. We now show that during inflammation, not only Paneth cells, but also other crypt and lamina propria cells show increased ER stress, which may reflect a general cellular stress response in the presence of pro-inflammatory cytokines or bacteria. Indeed, we demonstrate that stimulation of intestinal epithelial cells with bacteria or LPS triggers a significant upregulation of the ER stress marker GRP78. However, not all cell lines showed this effect, which may be a reflection of the genetic IBD risk factors present in these cell lines. Nevertheless, ER stress in both cell lines as well as organoids derived from IBD patients was reduced by treatment with Naltrexone, as were lamina propria GRP78 levels in biopsies from patients treated with Naltrexone, although this did not correlate with clinical response in all cases. Interestingly, genetic variants of the MOR gene OPRM1 affect response to high doses of NTX, however to what extent they may play a role in clinical and molecular response in IBD patients is as yet unclear[41].

Conclusion

In conclusion, our study provides additional insight into the mechanism of action of Naltrexone in intestinal inflammation, showing a direct effect of this opioid on intestinal epithelial wound healing and ER stress reduction. The clinical results are promising, and particularly given the low frequency and relative beneficial nature of side-effects, the use of LDN in therapy refractory IBD patients seems warranted. Future clinical research may also focus on the use of LDN earlier in the IBD treatment pyramid.

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Additional file 4.1 Material and methods

Organoid culture

Non-inflamed intestinal biopsies were collected from two IBD patients undergoing routine endoscopy for their disease. Organoids were collected in PBS and transferred into a 15 mL tube containing 10 mL complete chelating solution (CCS, MilliQ H_2O was supplemented with 1.0 g/L of Na2HPO4-2H2O, 1.08 g/L of KH2PO4, 5.6 g/L of NaCl, 0.12 g/L of KCl, 15 g/L of Sucrose, 10 g/L of D-Sorbitol and 80 lg/L of DL-dithiothreitol). Biopsies were washed three times by pipetting up and down 8–10 times. Biopsies were transferred into a 50 mL tube with 5 mL CCS and 100 uL 0.5M EDTA was added, then incubated on a rollerplate for 35 minutes at 4°C. Supernatant with EDTA was discarded and 5 ml fresh CCS solution was added. It was thoroughly suspended by pipetting up and down with 10 ml tip for 8–10 times to loosen crypts. Supernatant with crypts was transferred into a 15 ml tube and 2 ml FCS was added. Then, crypt suspension was centrifuged at 1200 RPM for 5 min. Supernatant was discarded and crypts were re-suspended in 12 ml cold advanced DMEM (Advanced DMEM/F12, 5 mL 100x GlutaMAX (GMX), 1% P/S, 500 µL Gentamicin and 5mL 1M HEPES) and centrifuged at 800 RPM for 5 min at 4°C. Crypts were suspended in 50 µL growth factor reduced phenol-red free Matrigel (Corning, Bedford, USA). Then, a 50 µL droplet of Matrigel/crypt mix was placed in the center of each well of a 24-well plate, and was subsequently incubated at 37°C with 5% CO2 for 15 min. 700 µL of culture medium was added per well. The culture medium was supplemented with CMGF-, 2% of B-27 supplements (Gibco, Grand Island, USA), 1% of N2 Supplements (Gibco, Grand Island, USA), 500 pg/L of EGF, 1 mM n-Acetyl Cysteine, 10 mM Nicotinamide, 0.5 µM A83-01 (TGF-b inhibitor), 3 µM SB202190 (p38 inhibitor), 20% (vol/vol) of R-Spondin 1 (conditioned medium), 10% (vol/vol) of Noggin (conditioned medium) and 50% (vol/vol) of Wnt3a (conditioned medium). Culture medium was refreshed every 3 days, and organoids were passaged every 7 days. Passaging of human organoids was done by solubilizing Matrigel and mechanically breaking up the organoids by passing through a 5 ml tip inserting a 200 µL tip, single cells were then transferred to fresh Matrigel. The passaging was performed every 5–6 days with a 1:3 split ratio. Each well contains 10 or more organoids.

Cell viability assay

Cell viability was assessed using MTT assays. Cells (10,000) were seeded in 96 wells plates, and upon adhesion were treated with different concentrations of Naltrexone (Sigma Aldrich, St Louis, MA). After 24h 48h and 72h, cells were incubated with 5mM MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Aldrich, St

Louis, MA) for 3h and colorimetric changes were measured using a microplate reader (Model 680XR Bio-Rad) at 490 and 595 nm.

Wound healing assay

In short, in scratch-wound assays, cell monolayers were scratched with a pipette tip, washed twice, and treated with 1 µM Naltrexone or vehicle control. Concentration Naltrexone used was based on *in vivo* dosages (4.5 mg per ±60 kg bodyweight). Photographs were taken (Axiovert200 M microscope; Carl Zeiss BV, Sliedrecht, The Netherlands) to analyze the percentage of open wound area at 24 h (ImageJ software; US National Institutes of Health, Bethesda, MD, USA). Experiments were performed thrice in duplicate, with two measure-sites per scratch.

Western blotting

Western blotting was performed as described[26], with modifications. HCT116 and CACO-2 cells were treated with Tunicamycin (2 µM) or *E. coli* (paraffin-fixed DH5α, 6.25 e5/mL) or Lipopolysaccharides (LPS, 10 µg/mL) in the presence or absence of 1 µg/mL Naltrexone. Organoids were treated with LPS (10 µg/mL) in the presence or absence of 1 µg/mL Naltrexone. Subsequently, cells were washed with PBS and lysed on ice in 300 µL 2x concentrated Laemmli buffer (100 mM Tris–HCl (pH 6.8), 200mM dithiothreitol, 4% SDS, 0.1% bromophenol blue, 20% glycerol, and 2% DTT) and boiled for 5 minutes at 95°C. Organoids were also washed with PBS, matrigel was dissolved and the organoids were released. This solution was transferred to a tube and centrifuged for 8 minutes at 800 RPM. Supernatant was removed and the pellet was resuspended in 150 µL Laemli buffer and boiled for 5 minutes at 95°C.
Cell extracts were resolved by SDS–PAGE and transferred to polyvinylidene difluoride membranes (Merck chemicals BV, Amsterdam, the Netherlands). Membranes were blocked in 50% odyssey blocking buffer (LI-COR Biosciences, Lincoln, NE) in PBS/0.05% Tween-20 and incubated overnight at 4°C with primary antibody. After washing in PBS-T, membranes were incubated with IRDye® antibodies (LI-COR Biosciences, Lincoln, NE) for 1 h. Detection was performed using Odyssey reader and analyzed using manufacturers software. Experiments were performed at least twice.

Immunohistochemistry

FFPE tissue sections were immunohistologically stained for GRP78. Briefly, 5µm sections were deparaffinized in xylene and rinsed through graded alcohols (100% alcohols (18:1:1 100% ethanol: 100% methanol: 100% isopropanol), a 95% solution of

the 100% alcohols, and a 80% solution of the 100% alcohols). Next, slides were rinsed several times with fresh deionized water, followed by another 5 minutes wash using fresh water. Antigen retrieval was performed by boiling the slides in 600mL of 10mM sodium citrate buffer, pH 6.0 for 15 minutes. Slides were cooled for 20 minutes and washed extensively in double-distilled H_2O and PBS. Endogenous peroxides were blocked by soaking slides in a PBS/3% H_2O_2 solution for 10 minutes at room temperature. Subsequently, slides were rinsed in PBS and blocked by incubating in 10% goat serum in PBS at room temperature for 1 h. Thereafter, tissue sections were incubated with GRP78 antibody (BiP, Cell Signaling Technology, Danvers, MA) diluted in blocking buffer (1:100) overnight at 4°C. Next, slides were rinsed again in PBS for 5 minutes each wash. Rabbit envision (DAKO, Heverlee, Belgium) was used as secondary antibody.

Reverse transcriptase polymerase chain reaction (rt-PCR)

We used rt-PCR to determine MOR expression on the IEC cell lines. RNA was isolated as described previously.[29] Briefly, RNA was isolated using a NucleoSpin® RNA kit (MACHEREY-NAGEL, Düren, Germany) and cDNA was synthesized using the TAKARA reverse transcription system (TAKARA BIO INC, Shiga, Japan) PCR was performed in a 25 µL reaction, using GoTaq polymerase and GoTaq Flexi buffer, 2 mM MgCl2 (Promega, Madison, WI), dNTP (0.5 mM each, Roche), 50 ng template and 0.5 nM of the following primers: Forward: 5'-GGAAGCCCTCCAGGTTCATT, Reverse 5'- GGTCTCTTCACTGGGCACTC. Ribosomal protein (*RP2*) primers were used as control: 5'-AAGCTGAGGATGCTCAAAGG, 5'-CCCATTAAACTCCAAGGCAA.

Additional 4.2

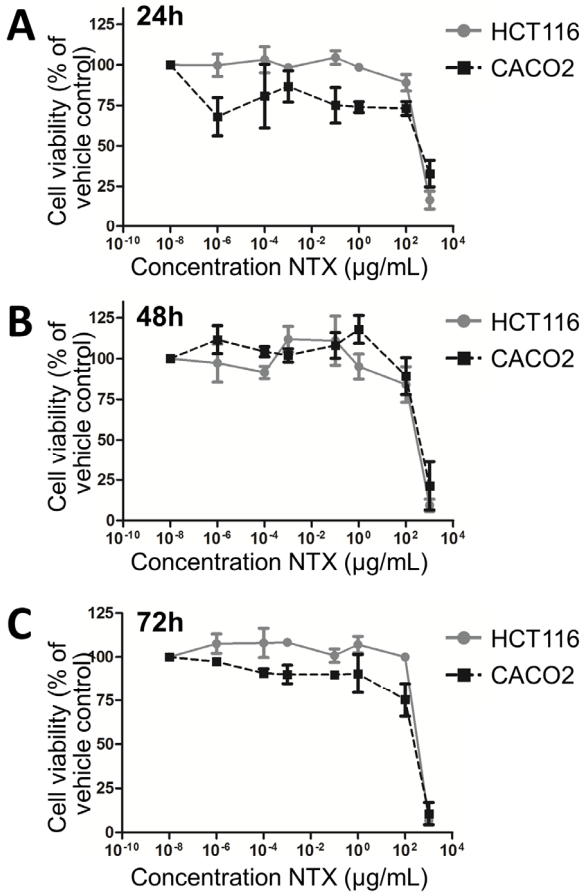

Figure S4.1

Additional files 4.3

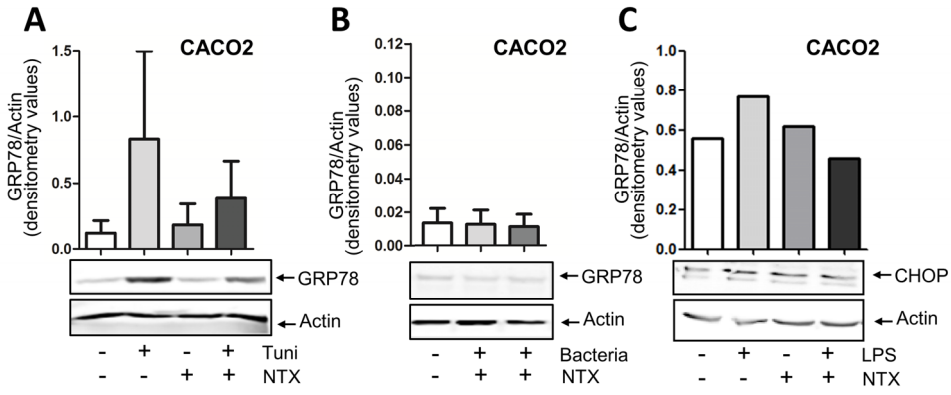

Figure S4.2

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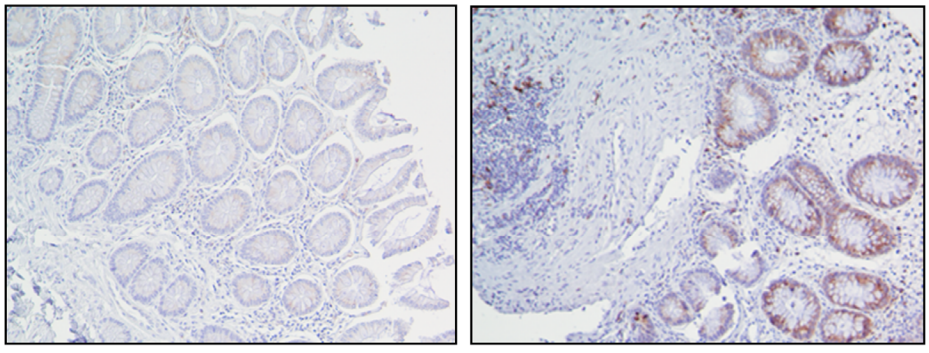

Figure S4.3

Chapter 5

Practical guideline for fatigue management in inflammatory bowel Disease

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Abstract

During active inflammatory bowel disease (IBD) fatigue is a common symptom, which seems related to active gut inflammation. However, even in remission, many patients suffer from fatigue that negatively affects quality of life and work productivity. Currently, robust knowledge on the pathogenesis and treatment of IBD-related fatigue is lacking. In order to alleviate the burden of IBD-related fatigue, a systematic approach is mandatory. We propose a fatigue attention cycle to enhance identification, evaluation and management of fatigued IBD patients. The benefits of the cycle are twofold. Firstly, it allows for systematic and uniform identification of patients with severe fatigue, in turn allowing for tailored non-pharmacological and pharmacological interventions. Secondly, uniform identification of such patients creates a well-defined patient base to investigate the underlying pathogenesis of fatigue, resulting in a greater understanding of this debilitating phenomenon and possibly resulting in the discovery of predictive factors and new treatment interventions.

Introduction

Fatigue is a common and aggravating symptom in patients with inflammatory bowel disease (IBD). IBD is characterized by chronic inflammation of the gastrointestinal tract with alternating periods of disease activity and remission, and mainly comprises two diseases: Crohn's disease (CD) and ulcerative colitis (UC). Many IBD patients suffer from additional symptoms that negatively affect their quality of life (QoL) and physical well-being, fatigue being an important contributor[1–3]. Fatigue is a common health problem in IBD, as illustrated by Cohen et al.[4], who showed that 25% of newly diagnosed IBD patients (n = 220) suffered from fatigue. Recently a pan-European online survey showed that over 80% of IBD patients suffered from debilitating fatigue[5]. Although fatigue understandably increases during periods of active gut inflammation, it nevertheless persists in half of the patients in whom sustained clinical and endoscopic remission is achieved and this ongoing fatigue affects both direct and indirect health costs[6-13]. In a systematic review, van Langenberg and Gibson[14] concluded that treatment of gastrointestinal symptoms has been extensively researched but subjective complaints, such as fatigue, have been ignored. Although patients highlight fatigue as a major concern, current literature on treatment strategies remains scarce. The causal relationship between fatigue and diminished QoL is not completely understood, though an intricate interaction of several factors, such as pain, psychological distress, sleep difficulties and ongoing inflammation, seems to be involved[15,16]. Consensus on the standard care for IBD-related fatigue, particularly regarding screening and management, is non-existent. Previous research by our study group has proved that psychotherapy has a positive effect on fatigue and QoL in patients with IBD, and, moreover, focuses on (existing) adequate coping strategies in these patients[17]. Following a pilot study, we performed a randomized controlled trial in fatigued IBD patients and showed that solution-focused therapy (SFT) significantly reduced fatigue. However, after stopping the therapy the fatigue seemed to reoccur. Our inability to adequately manage fatigue seems to be related to our limited knowledge of the pathogenesis of IBD-related fatigue[18]. In recent studies it has been hypothesized that upregulation of pro-inflammatory cytokines contributes to fatigue by directly inducing sickness behaviour, affecting muscle performance and indirectly affecting physical activity through sickness behaviour[13,19-23]. Moreover, different domains strongly associated with fatigue can be affected, such as physical, cognitive and emotional fatigue, and different, personalized strategies might be mandatory for accurate treatment of these different types of fatigue[19]. We sought to review available evidence in order to develop a clinically useful 'fatigue attention cycle' (Figure 5.1) regarding the evaluation and management of IBD-related fatigue with the aim of optimizing fatigue

care. The goal of the attention cycle is to identify patients suffering from fatigue and associated distress or interference with daily activities, in order to apply a systematic management strategy to alleviate fatigue and improve QoL.

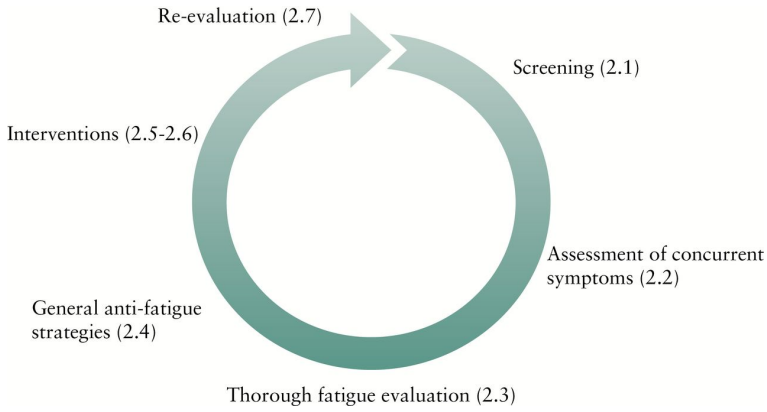

Figure 5.1 The fatigue attention cycle. All steps are explained in more detail in the corresponding paragraph in the text.

The fatigue attention cycle

The fatigue attention cycle is designed to aid clinicians in identifying and managing IBD-related fatigue. The cycle consists of 7 steps, elaborated in more detail below.

Screening

Screening for IBD-related fatigue is the primary step in the fatigue attention cycle. We propose the use of a visual analogue scale (VAS) with a score from 0 to 10 covering the severity of fatigue, with 10 representing severe fatigue and 0 representing no fatigue[20-22]. Because of the simplicity of the VAS, this first step should be easy to perform during routine outpatient visits. The purpose of this short screening method is to distinguish patients with mild fatigue (score 0-3), who require only basic education and counselling, from patients suffering from more severe fatigue (score 4-10), in whom a thorough and more focused fatigue evaluation is appropriate. These cut-off values are in line with the scores used in cancer-related fatigue[23].

Assessment of concurrent symptoms

In order to select an optimal treatment strategy, assessment of concurrent (treatable) symptoms is mandatory. Many factors are known to contribute to fatigue, such as inflammation, pain, emotional distress, sleep disturbance, anaemia, alterations in nutrition and overall nutritional status, and a diminished activity level. The presence of any of these conditions should be treated in accordance with practice guidelines and with referral to other (health-)care professionals as appropriate. Furthermore, medication side effects as well as alcohol and/or drug abuse should be taken into account, as should other comorbidities.

Inflammation

During periods of active gut inflammation, fatigue rates understandably increase[24-26]. Fatigue, on the other hand, is a highly reported symptom and a major concern in IBD patients and (chronic) fatigue can be a concealed indication of active disease. Disease activity should be ascertained by the usual clinical, radiological and endoscopic assessments in order to optimize treatment.

Anaemia

Studies have shown anaemia to be a common problem associated with fatigue in IBD patients. Anaemia can result from several deficiencies through several conditions, such as malabsorption, impaired dietary intake, suppression of iron binding and erythropoiesis or a combination of these factors[15,27,28]. Other causes of anaemia, specifically in IBD patients, include chronic intestinal bleeding, inflammation and certain types of medication. A systematic review performed by Goldenberg et al.[15] showed that isolated iron deficiency without the presence of anaemia is not a clinically relevant contributor to fatigue in IBD patients. Patients with established anaemia, regardless of the cause, should be thoroughly investigated and treated accordingly.

Nutrition

Various nutrient deficiencies have been associated with fatigue in the general population[29]. IBD subjects are at risk of nutrient deficiencies due to chronic inflammation, impaired muscle strength and malabsorption[30]. Even IBD patients that appear well nourished may actually harbour vitamin and/or mineral deficiencies. Vagianos et al.[27] showed a high prevalence of inadequate dietary intake of nutrients and biochemical deficiencies, such as vitamin B6 and B12, folate, ferritin and zinc, mainly in patients with active disease and vitamin D deficiency in both active disease

and remission. Nutrient status (ferritin, copper, zinc, folate, phosphate, magnesium, vitamin B6 and B12, calcium, vitamin D) should be restored if necessary, with referral to a dietician when appropriate.

Sleep disturbance

There seems to be a strong correlation between fatigue and sleep disorders, and problematic sleep quality seems common in active disease and also in non-active disease. Sleep disturbances have been associated with increased fatigue scores as well as deterioration of the inflammatory disease course, possibly through altered immune-endocrine factors associated with inflammation[16,31-33]. Although separate experiences, there is a strong correlation between fatigue and sleep, and diminished sleep quality should be considered and investigated in clinical practice, for instance using the validated Pittsburgh Sleep Quality Index (PSQI) questionnaire[16,34]. Cognitive behavioural therapy is considered the treatment of choice in adults with insomnia and has been proved effective in the long term[35-38].

Emotional distress

Psychological factors such as a depressive mood, stress, anxiety and impaired QoL are associated with fatigue[19,39]. Moreover, these psychological factors and diminished QoL are capable of negatively influencing the disease course of IBD[25,40,41]. It is difficult to measure the influence of psychological factors such as depression on (IBD-related) fatigue, since invalidating fatigue symptoms could be a cause of depressive symptoms as well[42]. Nonetheless, depression, anxiety and other psychological symptoms should be considered as part of this systematic approach to fatigue, as well as referral to a psychologist or psychiatrist.

Pain

In IBD, functional symptoms, including abdominal pain, have been shown to be associated with psychological dysfunction, even in quiescent disease[43-45]. Psychological factors may affect sensory processing and thus lead to variations in abdominal pain perception[46]. A simple VAS score can be used to determine the presence and severity of pain. Accurate assessment of the severity and origin of pain can contribute to appropriate diagnostic work-up and treatment.

Medication side effects

Although fatigue is noted as a possible side effect of practically all registered pharmacological drugs, review of current medication and recent changes in medication

is still important in the assessment of IBD-related fatigue. Of note, it has to be taken into account that fatigue has frequently been reported in pharmacological therapy for IBD, especially in those receiving anti-tumour necrosis factor α (anti-TNF) agents[24,47].

Activity level

Impaired muscle strength was seen in IBD patients compared with healthy controls[48-51]. Additionally, IBD-related fatigue was associated with a reduced physical activity level and decreased muscle strength[52]. Impaired physical activity and fitness must be considered in the assessment of fatigue-associated factors and insights into the activity level can contribute to the development of specific non-pharmacological treatment targets.

Comorbidities

Fatigue can be caused by a considerable number of other diseases. For instance, fatigue is associated with endocrine, renal, cardiac, respiratory and infectious diseases, amongst many others[14]. Specifically, other autoimmune diseases, such as rheumatoid arthritis, diabetes mellitus, coeliac disease, asthma and primary sclerosing cholangitis, should be taken into account, as IBD is associated with an increased risk of these autoimmune diseases. Comorbidities should therefore be considered as potential causes of fatigue.

Substance abuse

Alcohol and/or drug abuse are associated with emotional distress, poor sleep quality and anxiety, and should always be assessed in the work-up of IBD-related fatigue[53-55].

Thorough fatigue evaluation

Fatigue related to IBD is typically chronic and characterized by irreversibility[56,57]. It is not alleviated by rest and not related to exertion, and the compensation mechanisms that are useful in reducing acute fatigue are not effective in IBD-related fatigue. Screening for fatigue using the VAS described above will distinguish those patients with mild fatigue from patients suffering from moderate to severe fatigue. Since the latter group could benefit from a (non)-pharmacological intervention, a more elaborate assessment of the different areas of fatigue should take place. Multiple questionnaires have been used to assess fatigue in the literature. In a previous study by Tinsley et al.[3], the FACIT-F questionnaire, comprising 13 questions, was validated for IBD. Recently, the intricate concept of fatigue has called for a multidimensional assessment scale

designed to address the different domains of fatigue. The Multidimensional Fatigue Inventory (MFI-20[58]) and the Checklist Individual Strength (CIS) are 20-question, patient-reported, validated instruments for measuring motivation, activity level, concentration and, with the subscale CIS-fatigue, the severity of fatigue[17]. Similarly, Czuber-Dochan et al.[59] developed a questionnaire in collaboration with IBD patients for the assessment of fatigue and QoL, comprising 41 questions. Several studies report that the domains most strongly associated with fatigue are physical (functional, reduced activity), cognitive (mental, sensory) and emotional (affective, motivational, mood)[19,39,60]. Therefore, it is important to assess these domains with a questionnaire that reflects the multidimensional character of fatigue, in order to define specific treatment targets and their appropriate therapies.

General anti-fatigue strategies

Before proceeding to specific targeted interventions, more general anti-fatigue strategies should be employed. In particular, teaching patients how to plan their days seems to be the keystone in anti-fatigue strategies. Specifically, patients should be advised to distribute their energy throughout the whole day, to prioritize important events, to alternate their activities and to plan structured rests and breaks. Furthermore, relatives play an important role in the process of acceptance of fatigue, as their acceptance of and their support in managing disease-related symptoms, such as fatigue, are highly valued by IBD patients[61].

5. Non-pharmacological interventions

Non-pharmacological interventions, such as physical activity and psychosocial interventions, have been shown to help patients with a range of other chronic conditions to manage fatigue[19-62,63]. Several non-pharmacological interventions have been applied in IBD populations, mainly focused on mental health symptoms or overall QoL. However, literature on these interventions is conflicting. Some studies show beneficial effects of these interventions, such as stress management and cognitive behavioural therapy, mainly in the short term[64-66]. Unfortunately, evidence on non-pharmacological interventions in the treatment of IBD-related fatigue remains scarce. Garcia-Vega et al.[67] reported that adequate management of stress through stress-management techniques, had a beneficial effect on tiredness compared with treatment as usual in CD patients. Vogelaar et al.[68] showed that SFT, focusing on existing adequate coping abilities of patients for fatigue, had a positive effect on fatigue and QoL in IBD patients, but the effect diminished during follow up. Reduced activity and muscle strength was reported in fatigued IBD patients, with supporting evidence showing that

physical activity was beneficial for individuals with IBD by improving bone health, increasing muscle mass and function, increasing energy intake and possibly improving nutritional status. Additionally, QoL and fatigue were improved by exercise interventions in IBD patients[69-73]. Interestingly, studies in animal models have suggested that exercise may reduce the inflammatory response, thus providing benefits additional to those given by exercise[69,74]. However, it is challenging to specify guidance on the appropriate activity level or develop a practical physical activity protocol as the literature on these subjects in chronically ill patients is scarce[75]. Whether specific treatment combinations exist for specific types of fatigue, related to the various domains of fatigue (i.e. physical, cognitive, emotional), remains to be elucidated.

Pharmacological interventions

Currently, all pharmacological treatments for fatigue remain in the investigational stage. For instance, psychostimulants such as methylphenidate and dexamethasone have shown promising results in severe cancer-related fatigue[76-79]. However, these agents should be used cautiously and a specific treatment schedule has not yet been established, nor have these agents been investigated in IBD-related fatigue. Additionally, the randomized trials that have been performed in fatigued cancer patients have shown a significant placebo response[80]. Regarding IBD-related fatigue, few randomized trials have been performed. In a pilot study in 12 IBD patients, high-dose thiamine decreased overall fatigue scores. However, the included patients were poorly defined and did not have thiamine deficiency[81]. Other studies showed that the anti-TNF agents infliximab and adalimumab reduced fatigue, but these placebo-controlled studies were performed in patients with active disease[24,47,82,83]. Consequently, there is a clear necessity for studies on pharmacological interventions for the treatment of IBD-related fatigue in patients with quiescent disease.

Re-assessment

Given the fact that multiple factors influence (the severity of) IBD-related fatigue and that disease activity itself tends to fluctuate, the level of IBD-related fatigue is expected to change over time. Therefore, re-evaluating the presence and severity of fatigue is essential in optimal fatigue management.

Discussion

IBD-related fatigue is an underdiagnosed and undertreated phenomenon in current clinical practice with a severe negative effect on QoL, accompanied by high costs. At present, consensus on management of IBD-related fatigue has not been developed. A systematic approach to the assessment of IBD-related fatigue is of great importance in identifying and understanding this debilitating phenomenon and should start at the time of diagnosis of IBD.

The fatigue attention cycle as proposed ought to serve as an important tool for uniformly identifying severely fatigued patients and will aid in thoroughly evaluating and managing these patients. It is essential to consider these perspectives when engaging in strategies to optimize IBD care. Unfortunately, proper implementation of this cycle in clinical practice is hampered by lack of knowledge concerning the pathogenesis of IBD-related fatigue, making an effective treatment strategy very difficult to define. Currently, a non-pharmacological intervention (i.e. SFT), although not widely used, seems effective, although the effect diminished over time. Several studies on the effect of non-pharmacological interventions on overall QoL have been conducted, but their results are conflicting[64-66]. Furthermore, the duration of the beneficial effects of these interventions is generally limited. Consequently, there is a clear need for long-term studies involving well-defined non-pharmacological treatment interventions. Such studies would assess not only the short-term effects but also the long-term effects of these interventions, and eventually allow tailored management of fatigue. Pharmacological interventions are still in a premature state, and the lack of knowledge regarding the underlying mechanism of IBD-related fatigue makes the development of novel interventions complex. In order to discover important clues in the identification, prediction and treatment of IBD-related fatigue, the underlying biological mechanisms involved need to be unravelled. As proposed by van Langenberg and Gibson[14], pathogenic pathways involved in muscle fatigue and the contribution of disease-related psychological and neurobiological processes to fatigue genesis in IBD should be explored.

Uniform identification of patients with fatigue, via the proposed assessments, will be key in profiling patients in whom to study the pathogenesis of IBD. In these patients, it is important to focus on mediators of inflammation, but also on the keystones in the immuno-inflammatory response. In previous studies, differences in immune parameters (i.e. pro-inflammatory cytokines) between fatigued and non-fatigued patients were observed[84]. Studies on cancer-related fatigue have identified several pro-

inflammatory cytokines as being associated with the severity of fatigue[85,86]. Yet the exact mechanism by which fatigue is linked to the immune system and the reason why some patients but not others show such altered pro-inflammatory cytokine patterns during remission of disease remain obscure. As with other diseases, such as chronic fatigue syndrome (CFS) and multiple sclerosis (MS), the brain–gut axis is assumed to play a key role in the pathogenesis of IBD-related fatigue[15,87-91]. However, IBD is a chronic autoimmune disease affecting the gut, and therefore the effects of the brain–gut axis could be considerably different in IBD compared with CFS or MS. A combination of altered microflora, pro-inflammatory cytokine levels, increased stress (both hormonal and psychological) and a genetic predisposition may jointly underlie the mechanism of IBD-related fatigue. There is increasing evidence that the microbiota functions as a mediator in the bidirectional communication between the nervous system and the gut, though much remains to be discovered in this field[88]. Consequently, a 'back to basics' approach is required in order to identify mechanisms of this assumed multifactorial phenomenon of IBD-related fatigue. These insights may lead to the discovery of biomarkers to identify patients at risk of fatigue, and may eventually result in the development of an effective and efficient treatment strategy.

The recommendations presented here are largely based on expert experience and pragmatism and are supported where possible by published evidence. Key points include the need for the development of a well-defined fatigue attention cycle and structured support for IBD patients suffering from fatigue. We recognize that there may be large discrepancies between the recommendations presented here and those of allied healthcare professionals or IBD patients. However, these irregularities should not interfere with implementing a systematic diagnostic approach in clinical practice as a preliminary step towards proper identification and management of IBD-related fatigue.

Conclusion

IBD-related fatigue is a multifactorial debilitating phenomenon in need of a multidisciplinary approach. Current knowledge on the pathogenesis of IBD-related fatigue is severely lacking, resulting in sub-optimal identification and management of these patients. Uniform identification of fatigued patients will create a patient base in whom to study the underlying mechanisms of IBD-related fatigue. Research on the pathogenesis of IBD-related fatigue will allow the discovery of predictors of severe fatigue and the development of well-defined fatigue treatment algorithms and structured support for IBD patients.

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Chapter 6

Adalimumab in Crohn's disease patients: pharmacokinetics in the first 6 months of treatment

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Abstract

Background
Adalimumab (ADA) is an effective therapy for Crohn's disease (CD) patients. However, there is limited knowledge on the pharmacokinetic properties of ADA in CD patients.

Aims
To assess the pharmacokinetic properties of ADA in a retrospective clinical cohort of patients with CD, naïve to anti-tumor necrosis factor alpha therapy (anti-TNF).

Methods
In a single tertiary center, a clinical retrospective cohort was formed of 76 CD patients that started ADA treatment (160/80/40EOW) between July 2007 and September 2010. We serially evaluated ADA serum levels at week 0, 12 and 28.

Results
Patients were followed for a median time of 201 days (range 120-244) and received a median of 14 ADA injections (range 6-25). ADA levels, although divergent between patients, were stable between week 12 and week 28. There was no correlation between ADA level and time since last administration (r=-0.061). In a multivariable regression analysis of patient factors influencing week 28 ADA levels, the regression model containing CRP at week 28 and BMI at baseline weakly but significantly predicted week 28 ADA levels (R^2=0.193, P=0.004). Concomitant use of immunosuppressives was not a significant predictor (P=0.304).

Conclusions
Intra-individual ADA level seem very stable during the first 28 weeks of treatment, whereas inter-individual levels vary. ADA levels appear stable over a 2-week period, as the time since last ADA administration did not affect the ADA level. CRP and BMI weakly predict 28 ADA levels, whereas the use of immunosuppressives does not.

Introduction

The treatment of inflammatory bowel disease (IBD) has been revolutionized by the introduction of tumor necrosis factor alpha (TNF-a)-neutralizing medication[1-2]. Infliximab (IFX), a monoclonal chimeric antibody directed against TNF-a, is effective for both induction and maintenance therapy in patients with Crohn's disease (CD), including patients with draining perianal fistulas[3-8]. The development of antibodies to IFX and, as a consequence, low trough serum concentration of the drug have been implicated as predisposing factors for IFX treatment failure[9-12]. As such, recent studies have focused on using a combination of IFX levels and antibodies against IFX for treatment decisions, with varying results[13]. However, only limited data is available on therapeutic cut-off values in adalimumab.

Adalimumab (ADA), a recombinant human IgG1 monoclonal antibody against TNF-a is an established treatment for both IFX-naïve and IFX-exposed patients[14-17]. In one study, low trough serum concentration of ADA in CD patients was associated with increased early and late discontinuation rates, but no direct relationship between trough serum concentration and short-term efficacy of treatment was found[18]. However, this study was performed in patients who were intolerant or resistant to IFX therapy. Furthermore, in patients with rheumatoid arthritis, antibodies against ADA have been associated with low ADA trough serum concentration and decreased clinical response[19].

The primary aim of this study was to gain further insight on the pharmacokinetics of ADA, in a consecutive series of 76 patients with CD who were naïve for anti-TNF-a. At the group level, we examined changes in ADA level over time and assessed the correlation between ADA level and time since last administration of ADA. As an exploratory objective, we aimed to model the effect of different patient factors on the ADA level.

Materials and methods

Design and patients

A retrospective clinical cohort of 76 consecutive CD patients naïve to anti-TNF and who were starting ADA between July 2007 till September 2010 at the Erasmus MC, Rotterdam, The Netherlands, were included. Patients with tuberculosis, chronic

hepatitis B and/or C, or patients with immunodeficiency syndromes were excluded from the study.

Patients received ADA for induction of remission, steroid dependent disease, intolerance to previous therapies, fistula, extra-intestinal manifestations and as replacement for methotrexate in women wishing to conceive a child. All patients received a loading dose of 160mg and 80mg subcutaneously (sc) at week 0 and 2 respectively, followed by a maintenance dose of 40mg sc every other week thereafter. At week 12 all patients had received 6 administrations.

Patients were evaluated before starting ADA (week 0) and at week 4, 12, 20 and 28 after starting ADA. At each visit, the date of the most recent ADA administration was recorded, for calculation of the time since last ADA administration (i.e. the number of days between the ADA administration and the visit to the outpatient clinic).

ADA serum level measurements were performed at week 0, 12 and 28, by ELISA from peripheral blood, as described earlier[20]. Briefly, assay plates were coated with TNF-α and blocked with bovine serum albumin to prevent non-specific binding of ADA to the assay plates. Next, the plates were incubated with serum from peripheral blood. To determine the absolute levels, a standard curve of ADA concentrations was used. After washing the serum samples, the bound ADA was detected using an antibody-peroxidase conjugate directed against the Fc-part of IgG1, followed by an enzymatic color reaction. The assay was tested and validated using serum samples from patients receiving ADA and those who were naïve to these drugs. The detection threshold was 0.100µg/mL ADA.

Statistics

For the statistical analysis the SPSS 21.0 software package was used. Descriptive statistics were used to summarize the data. Medians with the range were calculated for continuous data and percentages were calculated for categorical data.

Apart from missing data, no adjustment for confounders was performed. Categorical data in unrelated groups were compared by the χ^2 test or Fisher's exact test, categorical data in related groups were analyzed by McNemar`s test. The Mann-Whitney test was used to compare continuous data. For paired test, the paired sample t-test was utilized. Pearson's correlation coefficient was used to assess the correlation of continuous

variables. For all these results, one or two-sided (as appropriate) P-values <0.05 were considered significant.

In the analysis of patient factors on ADA level, first the type of the distribution of the ADA level was determined using Kolmogorov-Smirnov's test (K-S test) and quartile-quartile plots (Q-Q plots). If ADA levels were found to be normally distributed, a linear regression model would be created and if the ADA level was found to be non-normally distributed, a logistic regression model would be created. After determination of the type of distribution, predictors were tested in univariable models. Additionally, a multivariable model was constructed, using the predictors with a P value of <0.20 in the univariable models, using stepwise backward elimination (probability of F to remove >0.10).

As for missing data, in case of missing CRP values at week 28, the values from respectively week 20 were carried forward. If these values were also missing, the case was excluded from the analyses involving CRP. Subjects with missing BMI values were excluded from analyses involving BMI.

Missing ADA levels were excluded from the ADA level analyses. If the time since last ADA administration was unknown, those ADA levels were excluded from the correlation analysis between time since last ADA administration and ADA level.

Ethical considerations

The study protocol was approved by the institutional review board and ethics committee of the Erasmus Medical Centre. All study procedures were conducted in accordance to the Declaration of Helsinki.

Results

Patient characteristics

Between November 2007 and September 2010, 76 anti-TNF naïve patients were started on ADA treatment (see Table 6.1 for patient details). Median follow up was 201 days (range 120-244 days) and patients received a median of 14 injections (range 6-25 injections) during follow-up.

Reasons for starting ADA therapy (Table 6.2) included active luminal CD, maintaining a steroid induced remission, step-up therapy due to intolerance or inefficacy of immunosuppressive therapy, fistulas, extra-intestinal manifestations or other reasons.

Table 6.1 Patient characteristics at baseline.

Number of patients	**76**
Male	33 (44.4)
Female	43 (56.6)
Mean age at diagnosis(years, range)	27 (11-74)
Mean age at start of adalimumab therapy (years, range)	39 (18-81)
Mean disease duration at start of therapy (years, range)	12 (0-49)
Montreal localization, n (%)	
L1 Ileitis	19 (25.0)
L2 Colitis	26 (34.2)
L3 Ileocolitis	31 (40.8)
Montreal disease behavior, n (%)	
B1 Non stricturing, non penetrating	42 (55.3)
B2 Stricturing	18 (23.7)
B3 Penetrating	16 (21.1)
Perianal involvement, n (%)	23 (30.3)
Current smokers, n (%)	20 (26.3)
Active fistulas, n (%)	8 (10.5)
Concomitant medication n (%)	
Corticosteroids	47 (61.8)
Immunosuppressives[a]	31 (40.8)
BMI kg/m^2, mean (SEM)[b]	24.1 (0.6)
CRP mg/L, mean (SEM)[b]	8.8 (1.9)
CDAI mean (SEM)[b]	161 (13.5)

BMI, body mass index; CRP, C-reactive protein; CDAI, Crohn's disease activity index. a: azathioprine, tioguanine or methotrexate. b=limited data, for BMI, n=60, for CRP, n=74, for CDAI, n=54.

Table 6.2 Reason for initiating adalimumab therapy.

Reason	**Number (%)**
Disease flare	49 (64.5)
Steroid dependent disease	12 (15.8)
Intolerance to previous therapy	5 (6.6)
Fistulas	4 (5.2)
Extra-intestinal manifestations[a]	4 (5.2)
Wish to conceive child[b]	2 (2.6)

a=arthralgia (n=3), pyoderma gangrenosum (n=1); b=used methotrexate prior to switch to adalimumab

Availability of data

Of the 76 patients included in the cohort, CRP values were available for 74 patients (with 17 data points carried forward). For ADA levels, data was available for 62, 65 and 57 patients at week 0, 12 and 28 respectively.

ADA levels over time start of therapy

Mean ADA serum concentrations were 9.5µg/mL (range 0.2-32.3) and 9,3µg/mL (range 0.2-24.4) at weeks 12 and 28 respectively (Figure 6.1, panel A), showing substantial inter-patient variance. At -0.47µg/mL (range -7.9-8.8µg/mL), the mean change in ADA level also showed a large inter-patient variance (Figure 6.1, panel B). There was no significant difference in the ADA level of week 12 compared with 28 (P=0.266).

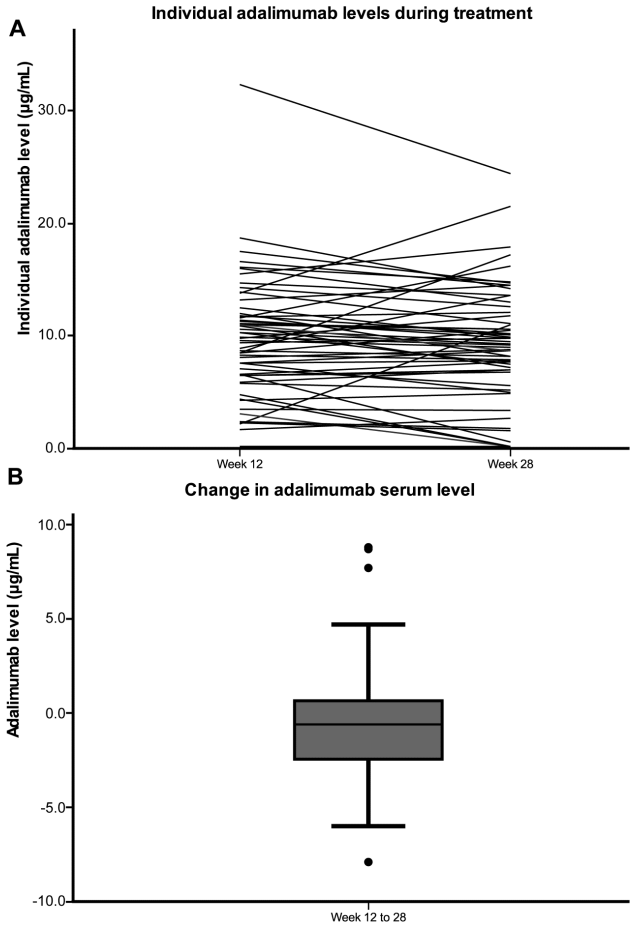

Figure 6.1 Panel A, adalimumab serum levels per individual, at week 12 and 28. Panel B, Tukey boxplot of change in individual adalimumab serum level from week 12 to 28. Error bars: 1.5 IQR (interquartile range).

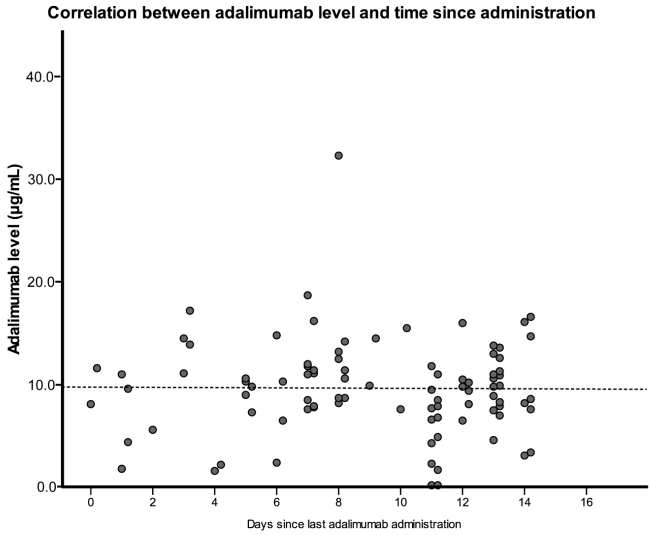

Figure 6.2 Adalimumab serum level and time since last adalimumab administration, (week 12 and week 28 results combined, N=92). The dotted line represents the linear correlation between the serum adalimumab level and the time since the last adalimumab administration (Pearson r=-0.016).

ADA levels and time since last ADA administration

In this clinical cohort, not all outpatient visits could be planned on the day before administration (i.e. 14 days since the last administration, essentially the "trough level day"). As such, we assessed the relation between the ADA level and the interval since the last administration. Figure 6.2 shows that there appears to be no correlation between ADA level and the time since last administration (r=-0.016).

Patient factors influencing ADA level at week 28

The ADA level at week 12 and 28 all had a normal distribution as shown by K-S test (P>0.200 and >0.200 respectively) and Q-Q plots. As such, a linear regression model was created to assess the influence of patient factors on the ADA level at week 28. The following patient variables were examined: age at baseline, BMI at baseline, CRP at week 28, gender, smoking behavior and use of concomitant immunosuppressive therapy at week 28 (Table 6.3). In the univariable analysis, age at baseline (P=0.016, adjusted R^2=0.085) and BMI (P=0.005, adjusted R^2=0.147, Figure 6.3) significantly predicted the ADA level at week 28. Of the other variables, only CRP at week 28 (P=0.113) reached the P<0.20 threshold for entry in the multivariable analysis. In the

multivariable analysis, both age at baseline and CRP at week 28 were no longer significant (P=0.304 and P=0.069, respectively). However, CRP remained in the model, as it did not reach the P>0.10 criterion for backward stepwise removal. As such, the final model contained BMI and CRP at week 28 as variables, though the model only weakly predicted the week 28 ADA level (full model, P=0.004, adjusted R^2=0.193).

Table 6.3 Results of univariable and multivariable analysis of patient factors influencing ADA level at week 28.

Variable	Univariable		Multivariable	
	Beta	P-value	Beta	P-value
Age at baseline	-0.319	0.016	-0.154	0.304
BMI	-0.408	0.005	-0.407	0.004
CRP at week 28 (or carried forward)	-0.212	0.113	-0.250	0.069
Gender	0.000	0.998		
Immunosuppressive therapy at week 28	0.137	0.344		
Smoking behaviour	-0.156	0.247		

Beta values are standardized. BMI, body mass index; CRP, C-reactive protein.

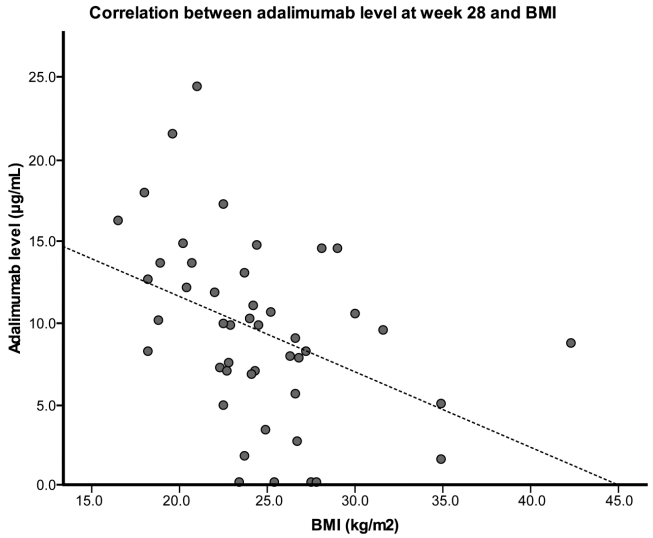

Figure 6.3 Adalimumab serum level after 28 weeks and BMI (N=46). The dotted line represents the linear correlation between the adalimumab serum level and the BMI (Pearson, r=-0.408).

Discussion

Our data shows intra-patient ADA levels are relatively stable, whereas inter-patient differences are substantial. Additionally, no correlation between ADA levels and the

time since last ADA administration was found. In our exploratory analysis of patient factors influencing ADA levels, a multivariable regression model containing CRP at week 28 and BMI at baseline weakly but significantly predicted week 28 ADA levels. We had initially hypothesized that specific patient-level factors would cause the majority of the variability in ADA levels. However, in the multivariable analysis only BMI and CRP significantly influenced the ADA level, accounting for just 19.3% of variability. As such, other unmeasured patient factors might play more important roles, such as the metabolism of immunoglobulins, which shows high inter-individual variability[25].

Our finding of stable ADA levels between week 12 and 28 is difficult to place into perspective using existing literature. The large CLASSIC-I, CLASSIC-II and CHARM[14-16] did not measure adalimumab serum levels, and other studies are of a cross-sectional design[21-24]. Karmiris et al.[18] find lower ADA trough levels than we do, but their study involves patients with previous exposure to infliximab. In another study with patients previously exposed to infliximab, Sandborn et al.[17] find levels similar to ours, but only measured these levels at week 4.

We found no relation between the ADA levels and the time since last ADA administration. Weisman et al.[26] performed a phase-1 pharmacokinetic study of ADA in rheumatoid arthritis patients. They show that the mean ADA half-life lies between 15 and 19 days after a single intravenous administration. Additionally, the same study shows that after 5 months of intravenous ADA treatment the mean half-life increases to 21 days. This is similar to data from original studies on gamma globulin metabolism, that report a mean a mean half-life of 23 days in healthy individuals[25]. Thus our findings of stable ADA serum levels, even 14 days after the last administration, could reflect this lengthened ADA half-life in our patients in the maintenance phase. If so, then increasing the time between ADA doses from two weeks to three weeks in these patients will probably not lead to a significant reduction of the ADA level, but should reduce the medication costs per patient. The clinical effects of lengthening the ADA dosing interval are unclear and could be the subject of future studies.

Our exploratory analysis on patient factors influencing ADA level showed a weak to moderate negative relationship with BMI. The relationship between BMI and ADA level was not seen in earlier studies, either because no modeling for factors influencing ADA level was performed[18] or BMI was not examined as a factor[27]. However, the relation between BMI and clinical efficacy has been examined. In the CLASSIC-II post hoc analysis[27], patients with a BMI greater than 29kg/m^2 were less often in clinical remission compared to patients with a lower BMI. Similarly, patients requiring ADA

dose escalation had a mean BMI of 25kg/m^2, compared to 23kg/m^2 in patients without dose escalation[28].

To our knowledge, even studies in other fields that use ADA therapy (e.g. rheumatic disease), the influence of BMI on ADA levels has never been examined[29-33]. As such, the negative correlation between BMI and ADA level requires confirmation in another study. In contrast, the use of immunosuppressive drugs had no significant influence in our model, similar to previous studies[18]. In univariable analysis, CRP was not a significant predictor of ADA level, whereas a weak negative correlation between CRP on ADA levels has been described previously[27]. However, with a P-value just above the 0.05 threshold, the addition of CRP in the multivariable analysis did result in a modest improvement of the model's accuracy.

We acknowledge the limitations of this tertiary referral center cohort, which could lead to bias. However, the patient characteristics seem to be an accurate reflection of the IBD population seen in general hospitals in the Netherlands[34].

The clinical nature of the cohort resulted in a variable schedule of our patients, causing patients to visit the outpatient clinic at different time points within their 14-day treatment schedule. As such we were unable to perform a trough level analysis. However, we feel that we have used this variability to our advantage, resulting in our analysis of ADA levels in relation to the time since last ADA administration.

Another limitation is the small sample size of our study. A larger study population would have made our results more robust and may lead to a different result of our multivariable analysis. However, in our opinion the drawbacks of a small sample size are offset by the benefits of the longitudinal design of our study. As mentioned before, previous longitudinal studies examining ADA efficacy in CD patients did not assess ADA levels , whereas more recent studies are of a cross-sectional design, providing no insight in the variability of ADA levels during treatment. The only other longitudinal study in adult CD that we are aware of was presented as an abstract and involved 22 patients. However, the abstract does not report the exact ADA levels[35].

The results of our study have a limited impact on clinical practice, as clinical effects and outcomes were not investigated. However, the great inter-individual differences in ADA level that we report here, underscores the need for an individualized approach to ADA therapy.

Given recent investigations into anti-drug antibody levels, measurement of levels of antibodies against ADA in this cohort could have been highly interesting. However, no reliable techniques for antibodies to ADA level measurements were available at time the cohort was followed, and no consent was acquired for post hoc measurements.

In summary, in this clinical cohort of anti-TNF naïve CD patients we show that ADA levels are relatively stable within a patient during the first 28 weeks of treatment. However, there is substantial variation between patients. Additionally, we find that a model consisting of CRP and BMI predicts ADA levels in a small but significant manner. Finally, we find that ADA serum levels are independent of the time elapsed since the last administration, possibly up to 21 days, which warrants further investigation.

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Chapter 7

Sex is associated with adalimumab side effects and drug survival in patients with Crohn's disease

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Abstract

Background

Adalimumab (ADA) is an effective treatment for Crohn's disease (CD). In rheumatology, sex differences concerning the response to ADA therapy have been described. However, such differences have not yet been reported for CD patients. As such, the aim of this study was to compare ADA treatment outcomes in male and female CD patients.

Methods

A clinical cohort was formed of consecutive CD patients starting ADA in a single tertiary center between March 2006 and February 2011. The cohort was followed up to August 2015. Clinical outcomes were primary non response, secondary non response and drug survival (ongoing ADA use). Reasons for stopping ADA were recorded. Kaplan-Meier analysis and Cox regression were used to assess drug survival.

Results

The cohort consisted of 107 female and 81 male patients. Median follow-up was 6.0 years (range 0.3-9.2). Drug survival was higher in male than female patients (48.1% vs 30.8%, P=0.016). Side-effects were reported more often by female patients (81.3% vs. 64.2%, P=0.008) and female patients ceased ADA more often due to side-effects (35.4% vs. 18.4%, P=0.017). In survival analysis, female sex was associated with higher cessation rates (P=0.006). Cox regression also identified female sex (P=0.020), along with higher baseline CD activity index (P=0.003), as predictors of ADA cessation.

Conclusions

Female sex is negatively associated with ADA drug survival. Female patients report more side-effects and cease ADA due to side effects more often. A more personalized and sex-specific approach seems warranted in order to increase drug survival in female patients.

Introduction

The treatment of inflammatory bowel disease (IBD), with its relapsing and remitting nature, has benefitted greatly from the introduction of antibodies against tumor necrosis factor alpha (TNF-α)[1,2]. Adalimumab (ADA) is a recombinant human IgG1 monoclonal antibody against TNF-α and is an established treatment for both anti-TNF-naïve and exposed patients[3-6]. When offered the choice, a substantial proportion of the patients with Crohn's disease (CD) prefer subcutaneously administered anti-TNF, such as adalimumab, over intravenous anti-TNF[7].

The now widespread use of anti-TNF agents has shifted the bulk of IBD healthcare costs from hospitalization and surgery to anti-TNF agents, with ADA making up 33.9% of all costs in CD patients[8]. Thus, it appears that ADA is commonly prescribed to CD patients, regardless of the sex of the patient.

However, it has previously been shown that the sex of the patient can have profound influences on drug metabolism and efficacy. For instance, in cardiovascular disease, the commonly used beta-blocker metoprolol has significantly higher drug concentrations in women than in men[9]. These differences are likely caused by sex specific differences in body composition (e.g. proportion of body fat) and drug metabolism (e.g. cytochrome P450 (CYP) enzyme activity). Though a biological agent such as ADA is not metabolized by CYP enzymes, sex-specific differences have been seen with biological drugs in other fields of medicine. Specifically, on a pharmacokinetic level several oncological biological agents such as bevacizumab, cetuximab and rituximab are cleared at different speeds between men and women[10]. Though the clinical implication of these pharmacokinetic differences are unclear, these differences again probably result from differences in body composition[11].

Of greater clinical relevance are observations in rheumatology, where female sex was found to be a significant negative predictor for longer ADA drug survival (i.e. the continued use of ADA). Particularly, lower drug survival was seen in women compared to men with arthritic psoriasis[12] and rheumatoid arthritis[13]. As such, the aim of this study was to investigate drug survival of ADA and possible other sex differences in a cohort of 188 CD patients.

Materials and methods

Design and patients

A prospective clinical cohort was formed, consisting of 188 consecutive CD patients that started ADA between March 2006 and February 2011 at the Erasmus University Medical Center Rotterdam, The Netherlands. Patients with evidence of tuberculosis, chronic hepatitis B and/or C, or patients with immunodeficiency syndromes did not start ADA and thus were not part of the cohort.

Of all patients in the cohort, the following parameters were documented: sex, age at diagnosis, age at start of ADA , Montreal disease classification, smoking behavior, body mass index (BMI), previous CD related medical history (i.e. CD related surgical history, previous CD related drug therapies) and ADA start and stop date.

Where available, clinical disease activity via the Crohn's disease activity index (CDAI) at start of ADA therapy, C-reactive protein (CRP) at start of therapy and ADA serum levels after 12 weeks of treatment were recorded. ADA serum levels were measured with ELISA, as described previously[14]. Patient reported side-effects were also recorded.

All patients received a loading dose of 160mg and 80mg subcutaneously (sc) at week 0 and 2 respectively, followed by a maintenance dose of 40mg sc every other week thereafter. ADA dose escalation occurred at the decision of the treating physician.

Patients were evaluated at the outpatient clinic at start of ADA therapy and at least every 4 months thereafter. In cases where ADA therapy was ceased, further follow-up and treatment at the outpatient clinic occurred according to treatment guidelines. CD related therapies initiated during and after ADA therapy were also recorded. Data was recorded up to the last outpatient visit preceding 01 August 2015.

The primary outcomes of interest was the ADA drug survival rate. Other outcomes of interest were sex specific differences in drug survival and adverse events. Drug survival was evaluated by determining maintained clinical response, primary non-response and secondary non-response. Primary non-response was defined as cessation of ADA therapy within 6 months due to lack of clinical improvement. Secondary non-response was defined as cessation of ADA therapy after at least 6 months of treatment (and thus, after an initial response), due to subsequent loss of response. Maintained response was defined as continued ADA therapy up to the end of follow-up. ADA cessation due to

other reasons, such as side-effects, was registered separately. Other outcomes of interest were the occurrence and outcome of dose escalation, reasons for stopping ADA and adverse events to ADA.

Statistics

For all of the statistical analysis the SPSS 21.0 software package was used. Descriptive statistics were used to summarize the data. Medians with range or means with standard deviations (SD) were calculated for continuous data as appropriate, and percentages were calculated for categorical data.

Variables were log-transformed as necessary and normality was assessed using Kolmogorov-Smirnov's test. Categorical data was compared via the χ^2 test or Fisher's exact test. The Mann-Whitney test was used to compare continuous data. For all tests, one or two-sided (as appropriate) P-values <0.05 were considered significant.

Ongoing ADA therapy was assessed using Kaplan-Meier analysis, with additional log-rank analyses to compare survival curves. Univariable Cox proportional hazard models were employed to assess associations between ongoing ADA therapy and patient factors. The assumption of proportional hazards was assessed visually, using a log(-log(survival)) versus log of survival graph. Additionally, a multivariable Cox proportional hazard model was constructed, using the predictors with a P value of <0.20 in the univariable models, using stepwise backward elimination (probability of F to remove >0.10). To address potential sex-specific effects, interactions between sex and significantly different baseline characteristics were also assessed in this model. The number of predictors entered in the multivariable model were limited to the number of events divided by 10.

Ethical considerations

The study was approved by the institutional review board and ethics committee of the Erasmus MC, University Medical Centre Rotterdam.

Access to Study Data

All authors had access to the study data and had reviewed and approved the final manuscript.

Results

Availability of data

Of the 188 patients included in this study, BMI values were missing in 4 patients, baseline CRP values in 24 patients, ADA serum levels in 58 patients and baseline CDAI values in 63 patients.

Patient characteristics

Between March 2006 and February 2011, 188 CD patients were started on ADA treatment (see Table 7.1 for patient details). Median follow up was 6.0 years (range 0.3-9.2 years). The total cohort consisted of 81 male and 107 female patients. At baseline, several sex differences were observed. Firstly, more peri-anal involvement was seen in male patients (48.1% vs. 33.6%, χ^2 P=0.044), whereas higher median CDAI values were found in females (174 vs. 154, Mann-Whitney-U P=0.036), with a trend for higher median CRP values in females as well (4.0mg/L vs. 3.0mg/L, Mann-Whitney-U P=0.079). When examining previous drug exposure, a significantly larger proportion of female patients had received either no treatment or only 5-ASA prior to start of ADA (13.1% vs. 1.2%, Fisher's exact test P=0.003), whereas a trend was seen for more prior immunosuppressive use in males (45.7% vs. 31.8%, χ^2 P=0.052).

Reasons for starting ADA therapy (Table 7.2) included active luminal CD, maintaining a steroid induced remission, step-up therapy due to intolerance or inefficacy of immunosuppressive therapy, fistulas, extra-intestinal manifestations and other reasons. Steroid intolerance was significantly more often a starting reason for males than females (9.9% vs. 1.9%, Fisher's exact test P=0.021).

Clinical outcomes to ADA therapy

Figure 7.1 displays the clinical outcomes to ADA therapy in this cohort, divided by male and female patients. In the first 6 months of treatment 31 patients stopped ADA therapy, with a significantly greater proportion of female patients than male patients stopping ADAA (21.5% vs. 9.9%, χ^2 P=0.034). Reasons for stopping were primary non-response in 13 patients, side effects in 15 patients and other reasons in 3 patients (1x chemotherapy for testis carcinoma; 2x physician decision due to ongoing systemic infection). There were no statistically significant sex differences concerning the reason for stopping ADA in the first 6 months of treatment. Finally, 2 patients were lost to follow-up within the first 6 months of treatment.

Table 7.1 Patient characteristics at baseline.

	Complete cohort	Males	Females	P†
Number of patients	188	81	107	
Median age at diagnosis (yr, range)	24 (5-74)	24 (10-74)	23 (5-63)	0.931
Median age at ADA start (yr, range)	36 (18-81)	36 (18-81)	36 (18-66)	0.201
Median disease duration at ADA start (yr, range)	9 (0-49)	10 (1-44)	8 (0-49)	0.532
Montreal localization, n (%)				
L1	44 (23.4)	22 (27.2)	22 (20.6)	0.290
L2	53 (28.2)	30 (28.40)	30 (28.0)	0.957
L3	91 (48.4)	36 (44.4)	55 (51.4)	0.345
Montreal disease behavior, n (%)				
B1	104 (55.3)	43 (53.1)	61 (57.0)	0.592
B2	46 (24.5)	20 (24.7)	26 (24.3)	0.951
B3	38 (20.2)	18 (22.2)	20 (18.7)	0.551
Perianal involvement, n (%)	75 (39.9)	39 (48.1)	36 (33.6)	**0.044**
Current smokers, n (%)	45 (23.9)	20 (24.7)	25 (23.4)	0.833
Drug use prior to ADA, n (%)				
None, 5-ASA and/or steroids	15 (8.0)	1 (1.2)	14 (13.1)	**0.003**
5-ASA, steroids and immunosuppressives	71 (37.8)	37 (45.7)	34 (31.8)	**0.052**
5-ASA, steroids, immunosuppressives and biologicals	102 (54.3)	43 (53.1)	59 (55.1)	0.780
Concomitant therapy, n (%)				
Corticosteroids	84 (44.7)	39 (48.1)	45 (42.1)	0.405
Immunosuppressives*	74 (39.4)	36 (44.4)	38 (35.5)	0.215
Previous intestinal surgery, n (%)	73 (38.8)	31 (38.3)	42 (39.3)	0.891
BMI kg/m^2, median (range)	23.5 (15.8-43.3)	23.3 (16.6-34.9)	24.1 (15.8-43.3)	0.172
CRP mg/L, median (range)	3.0 (1-99)	3.0 (1-89)	4.0 (1-99)	0.079
CDAI, median (range)	168 (10-689)	154 (12-298)	174 (10-689)	**0.036**

BMI, body mass index; CRP, C-reactive protein; CDAI, Crohn's disease activity index. *: azathioprine, tioguanine or methotrexate; †: P-value for comparison between male and female patients.

Table 7.2 Reasons for initiating adalimumab therapy.

	Total cohort (N=188)	Male (N=81)	Female (N=107)	P‡
Reason for initiating adalimumab	Number (%)	Number (%)	Number (%)	
Disease flare	141 (75.0)	58 (71.6)	83 (77.6)	0.350
Intolerance to previous therapy	20 (10.6)	7 (8.6)	13 (12.1)	0.440
Steroid dependent disease	10 (5.3)	8 (9.9)	2 (1.9)	**0.021**
Patient wish*	8 (4.3)	5 (6.2)	3 (2.8)	0.294
Fistula	6 (3.2)	2 (2.5)	4 (3.7)	0.701
Wish to conceive child†	3 (1.6)	1 (1.2)	2 (1.9)	0.999

*: patients in remission with infliximab who chose to switch to adalimumab; †: females in remission with methotrexate; ‡: P-value for comparison between male and female patients.

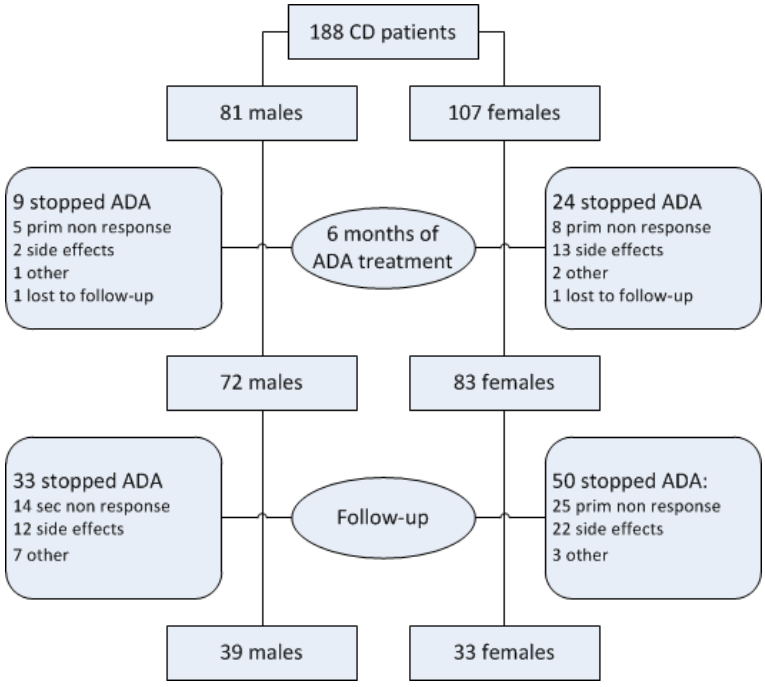

Figure 7.1 Flowchart depicting outcomes in the cohort, divided by male and female patients.

The remaining 72 male and 83 female patients achieved an initial clinical response. During follow-up, 83 patients of the initial responders stopped ADA therapy. A trend for the proportional difference in female and male patients stopping ADA was seen (60.2% vs 45.8%, χ^2 P=0.073). Reasons for stopping were secondary non-response in 39 patients, intolerance in 32 patients and other reasons in 12 patients (5x patient decision, 4x malignancy, 2x pregnancy, 1x suspected malignancy). No statistically significant sex differences in the reason for stopping ADA were observed amongst the patients with an initial response. At the end of follow-up, 39 male and 33 female patients still had a clinical response, which translates into a significantly greater maintained clinical response rate in male than female patients (48.1% vs. 30.8%, χ^2 P=0.016).

Similarly, in Kaplan-Meier analysis a difference was seen in male and female ADA continuation rates (Figure 7.2, log-rank P=0.006). Additionally, in univariable and multivariable Cox proportional hazard models (Table 7.3), male sex (beta=0.591, hazard ratio = 1.807, P=0.020) predicted longer ADA treatment duration. A lower CDAI at start

(beta=0.003, hazard ratio=1.003, P=0.003) was also predictive of longer ADA treatment duration. Previous anti-TNF exposure and steroid use at start of ADA therapy were removed from the multivariable model during the backward stepwise elimination. Of note, no interactions were seen between CDAI and sex or between peri-anal involvement and sex, despite the baseline sex differences in these two variables.

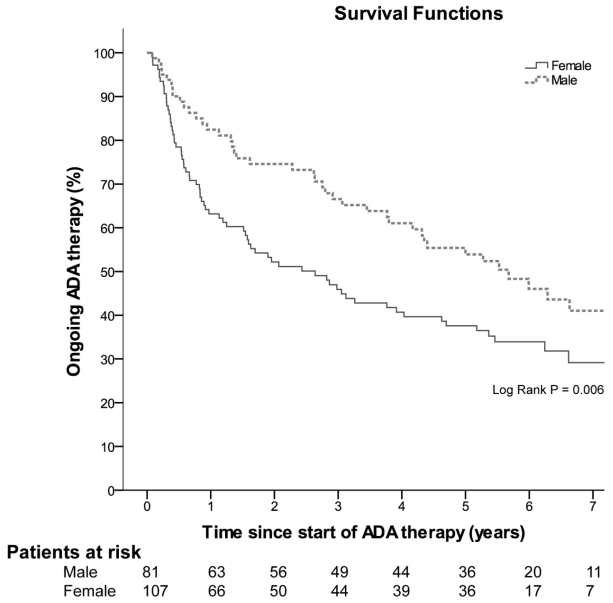

Figure 7.2 Kaplan-Meier survival curve, displaying ongoing adalimumab treatment in males and females, over 7 years. The difference between ongoing treatment in males and females is significant (log-rank P=0.006).

Table 7.3 Univariable and multivariable Cox proportional hazard regression model.

Variable	Univariable		Multivariable		
	Beta	P-value	Beta	P-value	Hazard ratio
Gender	.533	.007	.591	.020	1.807
BMI*	-.004	.827			
Age at baseline	-.009	.231			
Active smoker	-.039	.853			
CRP at start*	.005	.412			
CDAI at start*	.004	<.001	.003	.003	1.003
Previous anti-TNF exposure	-.316	.101			
Presence of peri-anal disease	-.045	.817			
Steroid use at start	.073	.189			
Immunomodulator use at start	-.014	.944			
Week 12 adalimumab serum level *	.019	.381			

anti-TNF = anti tumor necrosis factor; BMI = body mass index; CDAI = Crohn's disease activity index; CRP = C-reactive protein. *: missing data for BMI (4), CRP (24), ADA level (58) and CDAI (63).

ADA dose escalation

In total, 106 patients (56.4%) in the cohort had at least one episode of ADA dose escalation, including patients with escalation after ADA re-treatment. In total, 140 ADA dose escalations occurred in these 106 patients. The proportion of female and male patients receiving dose escalation was not significantly different (57.0% vs. 44.4%, χ^2 P=0.842). The median ADA treatment duration prior to escalation was 13 months (range 1-95 months), with a median escalation duration of 7 months (range 1-105 months). Dose escalation led to recapture of response in 99 of 140 escalations (70.7%), with a significantly greater success percentage in male than female patients (83.7% vs. 60.7%, χ^2 P=0.011).

Side-effects

The majority of patients reported side effects to ADA. Only 50 patients (26.6%) did not report any side-effects. In total, 254 adverse events were reported by 138 patients (Supplementary Table S7.1). Most commonly reported were injection site skin reactions such as erythema, bullae or hematomas (30), followed by respiratory tract infections (27), arthralgia (24) and generalized skin rash (23). Hair loss (16), fatigue (14), skin infection (14), nausea (13), dry skin (13) and headache (11) were also reported frequently.

Side-effects were more often reported in females than males (81.3% vs 64.2% respectively, χ^2 P=0.008). Additionally the median amount of reported side-effects was greater in females than males (2 vs. 1, Mann-Whitney-U P<0.001). During the whole follow-up period, female patients stopped ADA more often due to side-effects than male patients (35.4% vs. 18.4%, χ^2 P=0.017), whereas no significant differences were seen regarding stopping due to non-response or other reasons.

Regarding laboratory values, though mild liver enzyme abnormalities were seen in 123 patients, either these disturbances did not persist (69x), were present prior to ADA therapy (42x), or were clearly not caused by ADA (12x; e.g. due to thiopurine use, symptomatic cholelithiasis or post-surgery).

Discussion

In this clinical Crohn's disease cohort, we studied possible sex differences in the outcome to adalimumab treatment. At baseline, several differences already exist, chief

amongst them a difference in prior therapies. Additionally, we observe several sex differences concerning response, primarily a greater proportion of treatment in male than female patients, of 48.1% and 30.8% respectively. Survival analysis in this cohort also underscores the effect of sex on ongoing ADA treatment, along with baseline disease activity. Furthermore, we find that female patients report more side-effects and also cease ADA treatment more often due to side-effects than male patients.

As this study was a prospective cohort, several baseline differences exist between male and female patients. Importantly, there is a difference in therapies preceding start of ADA therapy. A significantly greater proportion of female patients were not treated with immunosuppressive drugs prior to the start of ADA. Though the reasons for not starting an immunosuppressive agent are unknown, possible explanations are a patient's fear of side-effects and the preference of the treating physician for top-down anti-TNF treatment.

To our knowledge, our observed sex difference concerning response to ADA therapy has not been previously described in CD patients, though it is in line with studies performed in rheumatology patients. One study in patients with psoriatic arthritis found that female sex was the strongest predictor of anti-TNF interruption in long-term anti-TNF treatment[11]. Similarly, a meta-analysis concerning patients with rheumatoid arthritis also reported that female sex predicts higher anti-TNF drug discontinuation rates[12]. In our study, the sex difference concerning response appears to be caused by differences in side-effects. In our whole cohort, 25.0% of the whole cohort stopped ADA due to side-effects, which is similar to previous studies[15-17]. However unlike the aforementioned studies, we find that the distribution of patients stopping due to side-effects is skewed toward female patients. However, a similar sex difference was seen in a study in 1009 IBD patients, of which 344 used anti-TNF (and 99 used ADA). In the whole anti-TNF subgroup, adverse reactions were seen more often in females than males, and females stopped anti-TNF treatment more often due to adverse reactions than males, but no statistically significant difference was seen within the subgroup of 77 patients using ADA[18].

A possible explanation for the sex differences in side-effects may lie in sex specific physiological differences. On average, women have a smaller organ size and a higher proportion of body fat than men. Additionally, in pre-menopausal women, the menstrual cycle results in fluctuations in the percentage of tissue-water. These physiological differences result in different drug distribution volumes, and could thus lead to different responses to drug therapy[19].

Additionally, modulation of CYP3A4 enzyme activity may also play a role. In general, women show higher CYP3A4 enzyme activity than men[20], probably due to sex related differences in sex hormone levels. However, CYP3A4 has been shown to be influenced by TNF-α[21], thus the use of an anti-TNF agent such as ADA may influence CYP3A4 activity. Via this route, ADA may influence the metabolism of other commonly used drugs, possibly causing patients to experience side-effects to these drugs, which in turn are attributed to ADA use. These effects may be further compounded by differences in perception of pain and adverse events in male and female patients[22].

Also, the clearance of ADA may differ between male and female patients. Mathematical modelling using data from ulcerative colitis patients receiving infliximab showed a 33% lower drug clearance in females than males[23]. Another modelling study amongst both CD and UC patients showed approximately 50% lower drug clearance in females than males[24]. However, it is unclear how this mathematical difference in infliximab clearance affects ADA serum levels, as previous reports did not find sex to significantly influence ADA serum level[25].

Furthermore, the difference in disease activity and previous at the start of ADA therapy suggests that female patients were slightly more ill at start of therapy, possibly reflecting reluctance to start anti-TNF therapy in female patients. Also, the sex difference in previous drug exposure is also suggestive of some form of bias against the prescription of immunosuppressive agents in general to female patients. However, the possibility of female patients being more ill at the start of ADA therapy does not readily explain the observed sex difference in side-effects occurring during the course of treatment.

Given the differences observed between men and women, it is conceivable that providing female patients with additional personalized information prior to the start of ADA therapy could reduce the observed sex-difference in reported adverse reactions. Specifically, providing more information concerning the possible side-effects may result in different patient expectations, which subsequently could reduce the high amount of drop-outs due to side-effects, as observed in our study.

The efficacy of ADA in our whole cohort was similar to the efficacy reported in previous studies. Our 6.9% primary non-response rate is comparable to the 9% lack of response rate reported in the open-label arm of the CLASSIC-II trial[26]. Similarly, the 27% discontinuation rate (for reasons other than lack of response) is comparable to the findings from our cohort.

In this cohort dose escalation to 40mg every week occurred in 56.4% of patients, with some patients receiving multiple periods of dose escalation. This proportion is similar to the reported escalation rates in other studies, which vary from 13.2% to 63.4%[27,28]. In our cohort, dose escalation was successful in 70.7% of attempts, similar to the reported success rate in other studies, which ranges from 36% to 86% in smaller studies[27,29], to 70 to 80%[30-32] as reported in larger studies.

Of note, we did not observe a sex difference in the need to dose escalate. This is similar to a study in 75 CD patients requiring dose escalation, though this study did find that male patients required dose escalation earlier than female patients[33].

This real-life tertiary referral cohort has several limitations. Though our cohort is certainly not the largest ADA cohort to be reported on, though our follow-up duration is relatively long. Also, the missing data at baseline may have influenced the results of the Cox analysis. Furthermore, the influence of CRP levels and CDAI scores during treatment could not be assessed, as these values were not regularly available during follow-up. Additionally, due to the tertiary nature of our hospital, the patients enrolled in this cohort may not accurately reflect clinical practice in smaller regional hospitals. Finally, we were unable to gauge the efficacy of ADA via an objective endpoint such as mucosal healing.

In summary, in this clinical cohort of CD patients treated with ADA, we reconfirm the overall efficacy of ADA for CD patients. Moreover, we show several gender differences in response to ADA therapy. Chiefly, the success rate of ADA therapy is higher in males than females, both for initial treatment, but also for dose escalation. The greater proportion of side-effects in females appears to be the major cause to the difference in drug survival. This suggests that a personalized approach to female patients starting ADA could positively influence the incidence and severity of side-effects and thus reduce the high drop-out rate seen in female patients.

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Supplemental table

Table S7.1

Adverse event	N (%)
Injection site skin reaction	30
Upper respiratory symptoms*	27
Arthralgia	24
Rash	23
Hair loss	16
Fatigue	14
Skin infection	14
Nausea	13
Dry skin	13
Headache	11
Eye symptoms†	9
Myalgia	6
Dizziness	6
Paresthesia	5
Vaginal mycosis	4
Pain extremities	4
Fever	3
Palpitaitons	3
Mood swings	3
Chest pain	2
Muscle cramps	2
Polyneuropathy	2
Urinary tract infection	2
Other‡	18
Total	254

Chapter 8

Patient sex does not affect endoscopic outcomes of biologicals in patients with inflammatory bowel disease, but is associated with adverse events

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Abstract

Background

Biological therapies are currently the mainstay in the treatment of patients with inflammatory bowel diseases (IBD). There are several known factors influencing the efficacy and tolerability of biological therapies, such as CRP levels at start of therapy or previous biological use. Whether patient sex affects the efficacy or tolerability is currently unclear, yet this knowledge would be helpful for risk and benefit stratification. This study assesses the role of patient sex on the efficacy and tolerability of biological therapies used for the treatment of IBD.

Methods

A systematic literature review was performed using Embase (including Medline), Medline OvidSP, Cochrane Central Register of Controlled Trials, Web of Science and Pubmed. The primary outcome was the influence of patient sex on endoscopic outcomes in IBD patients treated with biologicals. The secondary outcome was the influence of patient sex on adverse events during biological therapy. Studies examining either of the outcomes were included in the assessment, regardless of study type or setting.

Results

The search yielded 19461 citations, after review 55 studies were included in the study, involving 28465 patients treated with adalimumab, certolizumab pegol, infliximab or vedolizumab. Of the 41 studies that objectively examined patient sex and efficacy of biological therapy, none find a significant association. Of the 14 studies examining patient sex and adverse events, 7 find that adverse events such as infections or skin lesions occur more frequently in female than in male patients. No meta-analysis of the primary or secondary outcome could be performed due to lack of exact reporting of summary measures.

Conclusions

There is no evidence for a sex difference in endoscopically measured response to biological therapies in IBD patients. However, there is an influence of sex on the occurrence of adverse events.

Introduction

Due to their chronic nature the inflammatory bowel diseases (IBD), consisting of Crohn's disease (CD) and ulcerative colitis (UC), usually require life-long drug therapies. The treatment paradigm seems to switch and the current approach has been changed to a more accelerated step-up management of the IBD patient. Currently a large proportion of IBD patients are treated with biologicals, with studies reporting in the range of 20-25% in Western countries[1-3], and the use of biologicals seems to increase[3-4]. This increasing use necessitates the identification of factors predictive of drug efficacy and drug survival. Previously identified factors known to affect efficacy and tolerability of biological therapies in IBD patients include previous use of another biological drug[5], baseline C-reactive protein levels[6] and serum drug levels[7]. A simple factor to include in the treatment strategy could be patients' sex. Sex is already implicated as an important factor in the pathogenesis of IBD[8].

However, the current evidence on the role of patient sex on the actual response to biological therapies is conflicting. Several studies specifically report on differences in response and adverse events between male and female IBD patients treated with biologicals[9,10] whereas other studies report no significant differences between male and female patients[11,12]. Thus it remains unclear if a patients' sex plays a role in the efficacy or tolerability of biological therapies. This study aimed to systematically search the literature for evidence regarding the possible association of patient sex and biological therapies, concerning efficacy (measured objectively via endoscopy) and the occurrence of adverse events.

Objectives

To systematically review the literature for studies concerning established biological therapies for patients with inflammatory bowel disease, examining the possible influence of patient sex on:

- Objectively measured efficacy, defined as disease activity measured via endoscopy. Examples of this primary outcome include sigmoidoscopy, ileocolonoscopy and capsule endoscopy.
- Adverse events defined as any adverse event possibly related to biological use. Examples of this secondary outcome are infusion reactions, injection site reactions and hypersensitivity reactions.

Methods

Search strategy

A systematic database search was performed on 08 April 2019, without restrictions on language, publication year or publication status. The search was performed by librarians specialized in database searches. The search was performed in the following databases: Embase (including Medline), Medline OvidSP, Cochrane Central Register of Controlled Trials, Web of Science and Pubmed. The detailed digital search strategy is provided in the digitally available appendix.. Additionally, the reference lists of all potentially relevant articles were studied for further trials. Any studies found trough this search also had their reference lists studied.

Review and study selection process

Titles and abstracts identified through the search strategy were assessed by two independent reviewers (ML and EP) for potential eligibility, using pre-defined criteria as described in the appendix. Disagreements were settled in consensus and, if necessary, after discussion with a third independent reviewer (CW). The manuscripts deemed potentially eligible for inclusion were obtained for full text review. The full texts were assessed by the two independent reviewers, using pre-defined eligibility criteria as described in the appendix. Discussions with the third independent reviewer were used to resolve disagreements.

Data extraction

Data from the eligible studies was extracted using a standardized form by the two primary reviewers. Differences in the extracted data were resolved through consensus or, if necessary, discussion with the third independent reviewer. For each study, the following data was extracted:

1. Study type and methods (including study duration, loss to follow-up)
2. Participants (including age, disease type, duration of treatment prior to enrolment)
3. Interventions (including drug, dosage, duration, formulation)
4. Outcomes (including definitions of the primary and secondary outcomes).

Quality assessment

The risk of bias of included studies was assessed using either the Newcastle-Ottawa Scale (NOS) for cohort studies[13], or the Cochrane risk of bias assessment tool for

randomized controlled trials (RCT) and post-hoc analyses of RCTs[14]. The NOS ranges from 0 to 9, with 9 the best score and the lowest risk of bias. The Cochrane tool assigns low risk, unclear risk or high risk to randomization, allocation and reporting bias respectively. The assessments were performed by the two primary reviewers and in case of disagreement, consensus was found after discussion with the third reviewer.

Data synthesis and statistical analysis

Results are reported using the summary measure provided by the included studies (e.g. odds ratio (OR), hazard ratio (HR), difference in means) with the respective P-values and/or confidence intervals. If only proportions were reported, the OR was calculated.

For meta-analysis, where applicable, studies were pooled using a random-effects model, regardless of statistical heterogeneity. Heterogeneity was tested using the Chi-squared test, the I-squared test and visual inspection of forest plots. If heterogeneity was present, we attempted to investigate the cause thereof (such as methodological factors or the outcome assessment). In the case of high heterogeneity (I2>75%), studies were pooled only if the direction of their results was consistent. Subgroup analysis or meta-regression would be performed post-hoc, if sufficient studies were included for meta-analysis.

Results

Results of the search

The literature search performed on 08 April 2019 identified 19461 citations, of which 11049 remained after automatic removal of double entries (Figure 8.1). After reviewing title and abstracts 10771 manuscripts were considered irrelevant (e.g. did not study biological, case reports, abstract format only, in vitro study, see also Supplemental Table S8.1). This resulted in 278 potentially relevant studies. Examining the reference lists did not yield in additional potentially useful manuscripts. In total, 273 manuscripts were assessed completely for eligibility as 5 manuscripts could not be retrieved (Figure 8.1: flowchart). Of these 273 studies, 217 were excluded for various reasons. The remaining 55 studies were included in this review (Table 8.1 and 8.2)[7,9,15-67].

Primary outcome

In total, 41 studies were included studying the objectively measured efficacy of biologicals in 4736 patients[7,15,17-20,24-27,29-31,36-46,48,50-59,61,63-67]. Concerning methodology, 24 studies were retrospective[7,18,24,25,27,29,31,39,41-43,45,48,50-53,55-58,61,63,64], 10 were prospective cohorts[15,20,30,36-38,44,46,54,66], 3 were post-hoc analyses of RCTs[17,26,65], 3 were cross-sectional[40,59,67] and 1 study was a combination of a retrospective and prospective cohort[17] (Table 8.1).

The quality of the cohort studies was fair to good, with a median NOS of 7 (range 4-8), the risk of bias for the post-hoc studies was considered unclear (Supplemental Table S8.2a and S8.2b). Regarding the post-hoc studies, the study by Bouguen et al.[19] involves a RCT with low risk of bias, however the post-hoc nature increases the risk of reporting bias. Additionally this study used only a subset of the RCT population, creating an unclear risk of selection bias. The study by de Cruz et al.[26] involved an open label RCT, as such there is risk of allocation and performance bias, however the risk of detection bias was low as the endoscopic outcome was evaluated by blinded central readers. The post-hoc analyses by Watanabe et al.[65] was also based on an open-label RCT, therefore the study was at risk of allocation, performance and detection bias.

Studies examining one biological

Thirty studies examined only one biological[7,15,19,20,24,26,27,29,30,37,38,40-45,50,52-54,56-59,61,63,65-67], 9 studied adalimumab[26,37,40,43,52,58,59,65,67], 16 studied infliximab[7,15,17,19,20,30,38,41,42,44,53,54,56,57,61,63] and 6 studied vedolizumab[24,27,29,45,50,66]. The details concerning setting (e.g. retrospective, prospective), use (i.e. for induction, maintenance or post-operative prophylaxis), patients (e.g. CD or UC) and outcome measures (e.g. endoscopic remission) varied widely.

Adalimumab

There were considerable differences in study settings and methodologies in the 9 studies concerning adalimumab. 3 studies were cross-sectional[40,59,67], 3 were retrospective cohorts[43,52,58], two were post-hoc studies[26,65] and the last study examined a prospective cohort[37]. Nevertheless, all studies found that patient sex was not significantly associated with endoscopic outcomes, measured at variable time points (e.g. mucosal healing after 8-14 weeks[52] or mucosal healing after one year[65]).

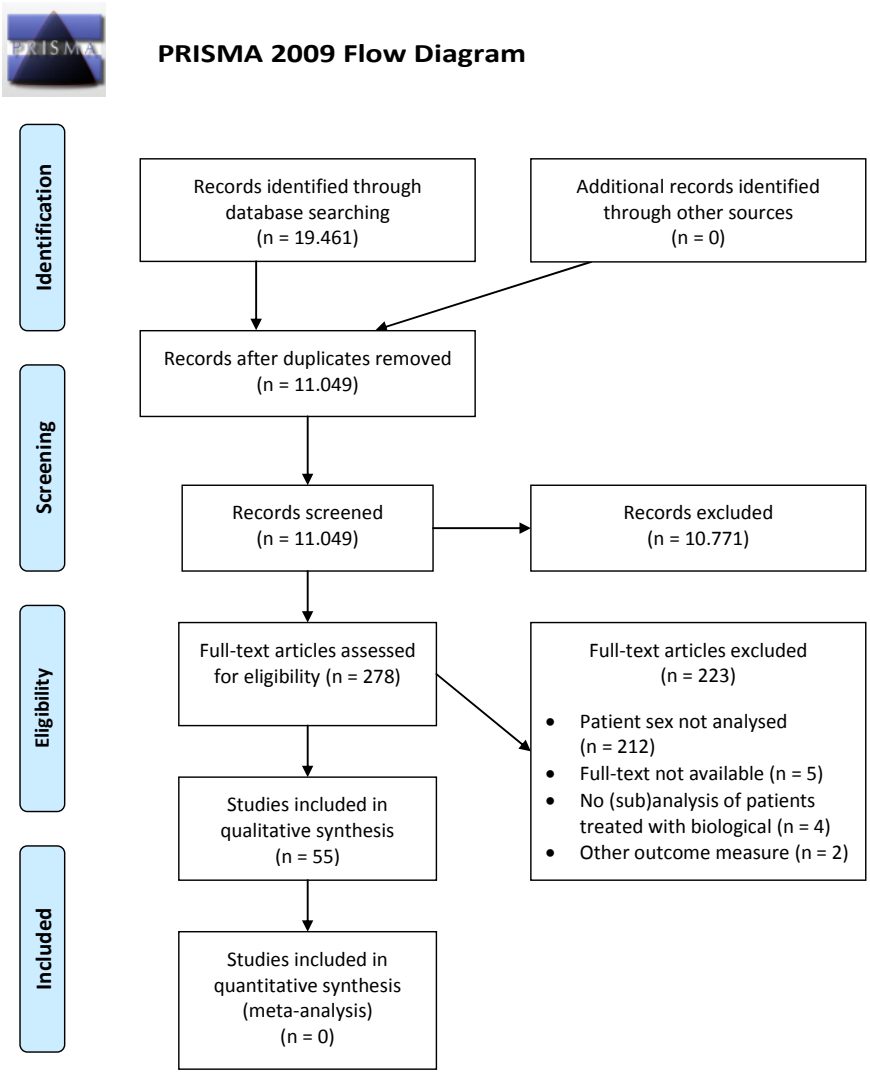

Figure 8.1 PRISMA flowchart of identification and selection of studies.

Table 8.1 Characteristics of included studies concerning patient sex and endoscopic efficacy. Grouped by biological studied.

Biological	Study type	Patients	Author, year	Outcome, measurement time point	Patient sex associated with outcome?
ADA, induction of remission	Prospective	43 CD	Hall, 2014	CECDAI, 52 weeks	Not associated
	Retrospective	201 UC	Kiss, 2014	MH, 12 months	Not associated
	Retrospective	43 UC	Papamichael, 2016	MH, 8-14 weeks	Not associated
	Retrospective	77 CD	Rismo, 2012b	MH, variable time-point	Not associated
	RCT post-hoc	135 CD	Watanabe, 2018	MH, 26 and 52 weeks	Not associated
ADA, maintenance of remission	Cross-sectional	98 IBD	Juncadella, 2018	CD: MH; UC: endoscopic Mayo ≤ 1	Not associated
	Cross-sectional	40 IBD	Roblin, 2014	CD: MH; UC: endoscopic Mayo ≤ 1	Not associated
	Cross-sectional	60 CD	Zittan, 2016	MH	Not associated
ADA, post-operative	RCT post-hoc	101 CD	de Cruz, 2015	Disease recurrence, 6 months	Not associated
	RCT post-hoc	84 CD	Taxonera, 2019	Disease recurrence, 52 weeks	Not associated
IFX, induction of remission	Prospective	285 UC	Arias, 2015	MH, 10-14 weeks	Not associated
	Combined†	126 UC	Armuzzi, 2013	MH, 12 weeks and 12 months	Not associated
	RCT post-hoc	508 CD	Bouguen, 2015	MH, 26 weeks	Not associated
	Prospective	30 UC	Brandse, 2015	Endoscopic Mayo decrease ≥ 1, 8 weeks	Not associated
	Prospective	63 UC	Farkas, 2016	MH, 14 weeks	Not associated
	Prospective	44 UC	Hassan, 2017	MH, 12 weeks	Not associated
	Retrospective	42 UC	Kelly, 2016	MH, 48 weeks	Not associated
	Retrospective	101 UC	Papamichael, 2016	MH, 10-14 weeks	Not associated
	Retrospective	49 UC	Ribaldone, 2018	Total Mayo decrease ≥ 3, 6 months	Not associated
	Retrospective	49 UC	Rismo, 2012a	Endoscopic Mayo ≤ 1, 8-12 weeks	Not associated
	Retrospective	97 CD	Shen, 2018	MH, 10 weeks	Not associated
	Retrospective	126 CD	Thomas, 2014	Complete / near complete MH, 12-20 weeks	Not associated
IFX, maintenance of remission	Retrospective	271 IBD	Kelly, 2017	CD: SES-CD < 3; UC: endoscopic Mayo ≤ 1	Not associated
	Prospective	35 CD	Koga, 2018	MH	Not associated
	Retrospective	110 CD	Papamichael, 2018	MH	Not associated
	Prospective	54 IBD	Paul, 2013	MH	Not associated

Table 8.1 (continued)

Biological	Study type	Patients	Author, year	Outcome, measurement time point	Patient sex associated with outcome?
VED, induction of remission	Retrospective	48 CD	Crowell, 2018	Undefined endoscopic improvement, 45 weeks	Not associated
	Retrospective	179 IBD	Dreesen, 2018	CD: MH, 22 weeks; UC: endoscopic Mayo ≤ 1, 14 weeks	Not associated
	Retrospective	212 CD	Dulai, 2016	MH, 6 and 12 months	Not associated
	Retrospective	222 IBD	Kotze, 2018	CD: MH or radiographic remission, 3, 6 and 12 months; UC: endoscopic Mayo = 0, 3, 6 and 12 months;	Not associated
	Retrospective	321 UC	Narula, 2018	Endoscopic Mayo = 0, 12 months	Not associated
	Prospective	82 IBD	Yacoub, 2018	CD: MH or radiographic remission, 12 months; UC: endoscopic Mayo ≤ 1, 12 months	Not associated
ADA, IFX, remission induction	Retrospective	248 IBD	Beigel, 2017	CD: SES-CD = 0; UC: endoscopic Mayo = 0; for both groups after median 11-25 months	Not associated
	Retrospective	48 UC	Dahlen, 2015	Total Mayo decrease ≥ 3, 14 weeks	Not associated
	Prospective	50 CD	Kuzela, 2012	Normal mucosal appearance via capsule endoscopy, 1 year	Not associated
	Retrospective	107 CD	Papaconsantinou, 2017	MH, 12-20 weeks	No associated
ADA, IFX, maintenance of remission	Retrospective	64 UC	Morita, 2017	UCEIS 0/0/0 or 1/0/0	Not associated
	Retrospective	145 IBD	Ungar, 2016	CD: SES-CD <3; UC: endoscopic Mayo ≤1	Not associated
ADA, IFX, post-operative	Retrospective	73 CD	Fay, 2017	Disease recurrence, after median 15 months	Not associated
	Retrospective	36 CD	Hiraoka, 2018	Disease recurrence, time not specified	Not associated
	Retrospective	44 CD	Preda, 2016	Disease recurrence, time not specified	Not associated
ADA, CZP, IFX, remission induction	Prospective	69 IBD	Guidi, 2014	CD: CDEIS <3, 1 year; UC: endoscopic Mayo ≤1, 1 year	Not associated

ADA, adalimumab; CD, Crohn's disease; CDEIS, Crohn's disease endoscopic index of severity; CECDAI, capsule endoscopy Crohn's disease activity index; CZP, certolizumab pegol; IBD, inflammatory bowel disease; IFX, infliximab; MH, mucosal healing; RCT, randomized controlled trial; SES-CD, simple endoscopic score for Crohn's disease; UC, ulcerative colitis; VED, vedolizumab; † = combined retrospective and prospective cohort.

Infliximab

Similar to the adalimumab studies, the 16 infliximab studies were varied in setting, scope and statistical methods. Of these studies, Papamichael et al.[7] found in univariable analysis that female UC patients were significantly more likely to achieve mucosal healing, measured 10-14 weeks after start of infliximab. However, this effect was no longer statistically significant in the corrected multivariable analysis. Similarly, all other infliximab studies found no significant association between patient sex and endoscopic outcomes, regardless of the statistical method employed.

Vedolizumab

The 6 studies examining patients using vedolizumab were more homogenous than the adalimumab or infliximab studies. Five of the vedolizumab studies were retrospective[24,27,29,45,50], and all 6 studies examined vedolizumab as remission induction. In the only prospective study by Yacoub et al.[66], in univariable analysis female IBD patients were significantly more likely to achieve mucosal healing after one year than male IBD patients, however in the corrected multivariable analysis, the difference between male and female patients was no longer statistically significant. The other vedolizumab studies also found no significant associations between patient sex and endoscopic outcomes.

Studies examining multiple biologicals

Of the included studies involving multiple biologicals, 7 examined a population treated with adalimumab or infliximab[15,25,31,39,46,48,51,55,64] and 1 concerned IBD patients treated with adalimumab, certolizumab or infliximab[36]. The first group of studies were all of a retrospective nature, with varying populations of CD patients, UC patients or both, as described in Table 8.2. The study concerning adalimumab, certolizumab or infliximab examined a prospective cohort of IBD patients.

Adalimumab and infliximab

Seven studies examined patients treated with either adalimumab or infliximab. All 7 studies were retrospective, but in varied patient groups and settings. None of the studies found a relation between endoscopic outcomes and the use of adalimumab or infliximab.

Adalimumab, certolizumab pegol and infliximab

Guidi et al.[36] assessed a prospective cohort of IBD patients treated with adalimumab, certolizumab pegol or infliximab for remission induction. Via logistic regression, no association was found between mucosal healing after 1 year and patient sex.

Meta-analysis

Several studies employed similar outcome measures (e.g. post-operative recurrence[31,39,55] or mucosal healing after 1 year[29,45,66]) and were thus suitable for meta-analysis. However, no meta-analysis could be performed due to lack of reporting of exact summary measures or the exact frequencies in which the outcomes of interest occurred in male and female patients.

Secondary outcome

In total 14 studies were included, assessing 17680 patients treated with biologicals[9,16,21-23,28,32-35,47,49,60,62]. Ten studies were retrospective[9,21,28,33-35,47,49,60,62], 1 was prospective16 and the remaining 3 were post-hoc analyses of RCT's[22,23,32] (Table 8.2).

The quality of the different studies was poor, with a median NOS of 5 (range 5-8). The 3 post-hoc studies were considered of low-risk of bias, as the original RCTs were of low risk themselves and the safety analyses were pre-specified and used the whole study population (Supplemental Table S8.2a and S8.2b).

Studies examining one biological

In total, 12 studies consisted of cohorts concerning a single biological[16,21-23,28,32-35,47,49,60]. Two studies involved adalimumab[23,47], 8 involved infliximab[16,21,28,33-35,49,60] and 2 assessed vedolizumab[22,32]. Of the adalimumab studies, 1 consisted of a cohort of CD patients[47] and the other of a cohort of IBD patients[23]. For infliximab, 7 studies were retrospective cohorts[21,28,33-35,49,60] and 1 was prospective[16]. The study populations consisted of CD patients in 2 studies[21,34] and IBD patients in 6 studies[16,33-35,49,60]. The remaining infliximab study involved mostly IBD patients but also included patients that used infliximab for rheumatologic or dermatologic diseases[28]. The 2 vedolizumab studies were both post-hoc analyses IBD patients treated with vedolizumab.

Table 8.2 Characteristics of included studies concerning patient sex and adverse events. Grouped by biological studied.

Biological	Study type	Patients	Author, year	Outcome	Patient sex associated with outcome?
ADA, induction of remission	Retrospective	188 CD	Lie, 2017	Any adverse event	More often in female patients (OR 1.27)
				Treatment withdrawal due to adverse events	More often in female patients (OR 1.93)
	Retrospective	5345 IBD	Colombel, 2018	Death (standardized mortality ratio)	Lower in male UC patients (ratio 0.38)
IFX, induction of remission	Prospective	810 IBD	Armuzzi, 2019	Serious adverse events	More often in female patients (HR 1.96)
	Retrospective	743 IBD	Fidder, 2009	Serum sickness-like disease, skin lesions	More often in female patients (OR 3.74 and OR 1.90)
				Mortality, neoplasia, serious infections, infusion reactions, auto-immune phenomena	Not associated
	Retrospective	336 IBD	Mourad, 2015	Any adverse event	Not associated
IFX, maintenance of remission	Retrospective	512 CD	Colombel, 2004	Serious infections	Not associated
	Retrospective	3161†	Ducharme, 2010	Any acute adverse drug reaction within 24 hours of IFX infusion	More often in female patients (OR 1.54)
	Retrospective	169 CD	Gonzales, 2017	Infusion reactions	Not associated
	Retrospective	197 IBD	Greener, 2018	Infections	Not associated
	Retrospective	100 IBD	Seiderer, 2004	Any adverse event	Not associated
VED, induction of remission	RCT post-hoc	2884 IBD	Colombel, 2017	Any serious infection	Not associated
	RCT post-hoc	2243 IBD	Feagan, 2018	Lower respiratory tract infection	More often in female patients (HR 2.11)
				Upper respiratory tract infection	Not associated
ADA, IFX, remission induction	Retrospective	149 CD	Teriaky, 2014	Any adverse event	Not associated
ADA, IFX, maintenance of remission	Retrospective	843 IBD‡	Zelinkova, 2012	Any adverse drug reaction	More often in female patients (OR 2.21)
				Treatment withdrawal due to adverse events	More often in female patients (OR 2.46)

ADA, adalimumab; CD, Crohn's disease; CZP, certolizumab pegol; HR, hazard ratio; IBD, inflammatory bowel disease; IFX, infliximab; OR, odds ratio; RCT, randomized controlled trial; UC, ulcerative colitis; VED, vedolizumab; † = of whom 1936 Crohn or ulcerative colitis. ‡ = of whom 150 used biologicals.

Adalimumab

Two studies were identified that examined patient sex and adverse events during adalimumab use. In a retrospective cohort of CD patients treated with adalimumab for remission induction, Lie et al.[47] described an increased frequency of adverse events reported by female patients compared to male patients (OR 1.27, P<0.01). Additionally, female patients reported adverse events as a reason for stopping adalimumab more often than male patients (OR 1.93, P=0.02). In a large post-hoc analysis of 16 RCTs and their open label extensions involving 5345 IBD patients, Colombel et al.[23] calculated standardized mortality ratio's and compared these to an age and sex matched control group. In this comparison, the standardized mortality ratio of male UC patients was lower compared to matched controls (ratio 0.38), but no statistically significant difference was found for female UC patients or male or female CD patients.

Infliximab

Eight studies described adverse events during infliximab use and patient sex. Three studies found significant associations, with Armuzzi et al.[16] describing a prospective cohort of 810 Italian IBD patients who started treatment with the infliximab biosimilar CT-P13, both for remission induction and for maintenance of remission. In this cohort serious adverse events occurred less frequent in male IBD patients than IBD female patients (HR 0.51, CI 0.35-0.76, P=0.001). In a large retrospective study involving 3161 patients treated with infliximab, Ducharme et al.[28] examined adverse events. However, in this large cohort 55% of patients received infliximab because of IBD, but the remainder was treated with infliximab because of rheumatologic or dermatologic conditions. Nevertheless, within this heterogeneous group of diseases an acute drug reaction (i.e. and adverse event within 24 hours of the infliximab infusion) was more likely to occur in female patients than in male patients (OR 1.54, P<0.001). Unfortunately no sub-analysis was performed to assess if this association remains in only IBD patients. Fidder et al.[33] retrospectively compared a cohort of 743 IBD patients treated with infliximab for remission induction with 666 IBD patients without exposure to biologicals. Serum sickness-like disease occurred more frequently in female patients than in male patients (OR 3.74, P<0.01). Skin lesions were also reported more often in female patients than in male patients (OR 1.90, P<0.01). However no sex difference could be detected for mortality, neoplasia, serious infections, infusion reactions and auto-immune phenomena. The 5 other studies found no association between patient sex and adverse events during infliximab use.

Vedolizumab

Two studies examined the possible role of patient sex on the occurrence of adverse events during vedolizumab therapy. In a post-hoc analysis of the GEMINI-1, GEMINI-2 and GEMINI open-label extension trials, Feagan et al.[32] examined the occurrence of respiratory tract infections in IBD patients treated with vedolizumab. They found that lower respiratory tract infections are more likely to occur in female patients than in male patients (HR 2.11, P=0.03). This effect was only seen in UC patients, not in CD patients. Furthermore, no association between patient sex and upper respiratory tract infections was found. A general analysis of safety of vedolizumab was performed by Colombel et al.[22] using post-hoc analysis of data from the GEMINI-1, GEMINI-2, GEMINI-3 and GEMINI open-label extension trials. In this study, patient sex was not found to be a significant risk factor for the occurrence of serious infections. Patient sex was not studied in analyses of other types of adverse events.

Studies examining multiple biologicals

Adalimumab and infliximab

In total, 2 studies were identified that examined the role of patient sex on adverse events during the use of adalimumab or infliximab[9,62]. One study found a significant associations between patient sex and adverse events. Zelinkova et al.[9] examined adverse events in a retrospective cohort of 843 IBD patients. In separate analyses of 150 patients treated with adalimumab or infliximab, adverse drug reactions were found to occur significantly more frequently in female patients than in male patients (OR 2.21, P=0.01). Further sub-analyses per drug revealed similar associations, though the association in adalimumab users was not statistically significant, possibly due to low patient numbers. Of note, this study also found that female patients stopped anti-TNF treatment more often than male patients due to adverse drug reactions (OR 2.46). The other study by Teriaky et al.[62] also examined a cohort of CD patients treated with adalimumab or infliximab, but found no association between the patient's sex and the occurrence of adverse events.

Meta-analysis

The outcomes examined in the included studies were considered too heterogeneous for meta-analysis. Additionally, similar to the studies concerning the primary outcome, the exact summary measures or the exact frequencies in which the outcomes of interest occurred in male and female patients were not reported.

Discussion

The objectives of this review were to assess the possible influence of patient sex on biological therapies, on endoscopic outcomes and adverse events. To our knowledge, this was the first systematic review investigating this research question. With regards to efficacy, none of the studies found an association between patient sex and endoscopically measured efficacy of biological therapies. As for adverse events, half of the included studies found an association between patient sex and various adverse events, with all these studies suggesting that these events occur more frequently in female patients.

The intention of this study was to perform a meta-analysis of the included studies, however several factors precluded synthesis of the data via meta-analysis. Firstly, outcome measures varied amongst studies, with the definitions of adverse events varying from 'any adverse reaction' to 'severe infections'. Secondly, the time-point at which outcomes were measured differed amongst the studies. Thirdly, the study populations were heterogeneous, with some studies examining biological naïve patients and others biological experienced patients or post-operative patients. Fourthly and most importantly, many studies simply reported that patient sex was not associated with the studied outcome, but without providing exact summary measures (e.g. odds ratio, difference in means) or the exact frequencies in which the outcome occurred in male and female patients separately. This prevented us from calculating summary measures to perform meta-analysis.

Pharmacokinetic studies in IBD patients concerning infliximab[68,69] and vedolizumab[70] reported a sex difference, regarding clearance and distribution volume. Similarly, in adalimumab a sex difference for apparent clearance has been reported in rheumatoid arthritis patients[71], but the kinetics have not yet been studied in IBD patients. Based on these preliminary studies it could be hypothesized that sex differences both in efficacy and adverse event rates could be present in IBD patients treated with biologicals.

However, we found no evidence for a sex difference in objectively measured endoscopic disease outcomes. This strongly suggests that biological therapies are effective regardless of patient sex, probably because the underlying inflammatory pathways affected by these therapies are not significantly different between female and male IBD patients. The lack of a sex difference in efficacy of biologicals is also seen in rheumatology patients[72,73] and dermatology patients[74,75] treated with anti-TNF agents.

Nevertheless, there have been consistent reports of a sex difference concerning decreased drug survival in female IBD treated with biologicals[76,77]. However if the efficacy of biologicals is similar in men and women, as shown by this review, this strongly suggests that factors other than primary non-response are responsible for the decreased drug survival. In populations that are not treated with biological therapies, there is literature suggesting increased rates of adverse events in females. In a large safety analysis of 7 observational studies (none in IBD patients), female sex was associated with the increased occurrence of side effects[78]. A similar result was found in a study regarding hospital admissions[79], wherein female patients were significantly more frequently admitted due to adverse drug reactions than male patients. Therefore a possible cause of decreased drug survival could be sex differences in adverse events. The results of this systematic review however are ambiguous. Though 7 studies did find that female sex is associated with adverse events during biological therapy, the other 7 included studies found no such association.

There are several limitations to this study. Concerning the primary outcome of objectively measured efficacy, the included studies varied greatly in their outcome measures. For instance, in CD patients some studies used SES-CD whilst others used CDEIS, and in UC patients some studies used the endoscopic Mayo score whilst others used UCEIS. Furthermore, even amongst studies using the same outcome measure, the definitions of response and remission could vary. Additionally, there was great variation in the timing of the endoscopic assessment across the included studies. Though this issue was identified during the review, it was decided to include all studies regardless of the heterogeneity of the outcomes. Though a more stringent set of inclusion criteria regarding endoscopic outcomes would have reduced heterogeneity, it was decided to be as inclusive as possible in order to detect a potential signal concerning sex differences. Furthermore given the lack of meta-analysable results, using more stringent criteria would not have resulted in a different conclusion.

The issue of high heterogeneity also occurs in the studies included for the adverse event analysis. Similar to the primary outcome, it was decided to use broad inclusion criteria in order to detect a potential signal concerning sex-differences in the occurrence of biological related adverse events. However, of the 7 studies that report a sex difference, in 3 studies the relation between the analysed adverse events and the drug used is debatable. Firstly, Colombel et al.[23] find a lower standardized mortality ratio in male IBD patients treated with ADA, but a direct causal relationship between ADA use and mortality seems unlikely. Similarly the adverse events analysed by Lie et al.[47] and Armuzzi et al.[16] include events probably related to biological use (e.g. injection

site reactions, infusion reactions) but also events that are likely unrelated to therapy (e.g. nausea, hair loss, headache). If the analyses in these studies were performed using only adverse events probably related to biological use, the results might no longer be statistically significant. In contrast, the other 4 studies that identify a significant sex difference specifically analyse events that are possibly therapy related, such as infusion reactions, serum sickness, respiratory tract infections and allergic-type reactions.

This ambiguity is also present in patients treated with biologicals for dermatologic or rheumatologic conditions. For instance in psoriasis patients some studies reported more adverse events in female patients[80,81] whereas other studies did not find this association[82]. Similarly the retention rates of biologicals in psoriasis patients was found to be associated with female sex in some studies[83,84] but not in others[85,86]. The same holds true in rheumatology patients treated with biologicals. Several studies reported an association between patient sex and adverse events[87] and drug retention rates[88,89], whereas other studies found no such associations[90].

In summary, this systematic review finds no evidence for differences in efficacy of biological therapies in female or male IBD patients, as judged endoscopically. Therefore the sex of the IBD patient need not be directly taken into account when considering starting biologicals or optimization of biological trough levels. The results concerning adverse events are ambiguous, with half of the studies finding an increased occurrence of adverse events in female patients treated with biological therapies, whereas the other half does not. Extra vigilance and proper counselling for treatment emergent adverse events might be warranted. Further investigations of possible sex differences in the occurrence and severity of adverse events could result in more accurate personalized medicine.

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Supplementary data

Table S8.1 Studies excluded (with reasons)

Studies included for detailed review	**278**
Exclusion reason	**Number of refs excluded**
Not full study (e.g. conference abstract, letter, comment, protocol)	1.406
Case report or case series	1.037
Not IBD (e.g. psoriasis, rheumatoid arthritis)	1.014
Not intervention of interest (e.g. azathioprine, lenalidomide, apheresis)	1.026
Other study group (e.g. children)	699
In vitro research / animal study	842
Not outcome of interest (e.g. cancer risk, PROM, radiology)	1563
Not available in English	106
Review or meta-analysis	2.966
Manually deduplicated	112
Total	10.771

Table S8.2a Quality assessments. Cohort studies assessed using Newcastle-Ottawa Scale Sorted, by outcome (efficacy studies, then adverse event studies) and alphabetically by author name.

Author, year	Cohort selection			Comparability		Outcome			Total score
	Selection of exposed cohort	Selection of non-exposed	Ascertainment of exposure	Outcome not present at start	Comparability of cohorts	Outcome assessment	Sufficient follow-up	Adequacy of follow-up	
Efficacy studies									
Arias, 2015	1	1	1	1	2	0	1	0	7
Armuzzi, 2013	1	1	1	1	2	0	1	0	7
Beigel, 2014	0	1	1	1	2	0	1	0	6
Brandse, 2015	1	1	1	1	0	0	1	1	6
Crowell, 2018	1	1	1	1	0	0	1	0	5
Dahlen, 2015	1	1	1	1	0	0	1	1	6
Dreesen, 2018	0	1	1	1	2	0	1	1	7
Dulai, 2016	1	1	1	1	2	0	1	0	7
Farkas, 2016	1	1	1	1	0	0	1	0	5
Fay, 2017	0	1	1	1	0	0	1	0	4
Guidi, 2014	1	1	1	1	2	0	1	1	8
Hall, 2014	0	1	1	1	0	0	1	0	4
Hassan, 2017	1	1	1	1	2	0	1	1	8
Hiraoka, 2018	0	1	1	1	2	0	1	1	7
Juncadella, 2018	0	1	1	1	2	0	1	1	7
Kelly, 2016	1	1	1	1	2	0	1	1	8
Kelly, 2017	1	1	1	1	2	0	1	1	8
Kiss, 2011	1	1	1	1	2	0	1	0	7
Koga, 2018	0	1	1	1	2	0	1	0	6
Kotze, 2018	1	1	1	1	2	0	1	0	7
Kuzela, 2012	1	1	1	1	0	0	1	0	5
Morita, 2017	0	1	1	1	0	0	1	1	5
Narula, 2018	1	1	1	1	2	0	1	1	8
Papaconstantinou, 2017	0	1	1	1	0	0	1	1	5
Papamichael, 2016	0	1	1	1	2	0	1	1	7
Papamichael, 2016	0	1	1	1	2	0	1	1	7

Table S8.2a (continued)

Author, year	Cohort selection			Comparability		Outcome		Total score
	Selection of exposed cohort	Selection of non-exposed	Ascertainment of exposure	Outcome not present at start	Comparability of cohorts	Outcome assessment	Sufficient follow-up	Adequacy of follow-up	
Papamichael, 2018	0	1	1	1	2	0	1	1	7
Paul, 2013	1	1	1	1	2	0	1	1	8
Preda, 2016	1	1	1	1	2	0	1	0	7
Ribaldone, 2018	1	1	1	1	2	0	1	0	7
Rismo_b, 2012	0	1	1	1	2	0	1	0	6
Rismo_a, 2012	0	1	1	1	2	0	1	0	6
Roblin, 2014	0	1	1	1	2	0	1	1	7
Shen, 2018	0	1	1	1	2	0	1	1	7
Thomas, 2014	0	1	1	1	0	0	1	1	5
Ungar, 2016	0	1	1	1	2	0	1	1	7
Yacoub, 2018	1	1	1	1	2	0	1	1	8
Zittan, 2016	1	1	1	1	2	0	1	1	8
Adverse event studies									
Armuzzi, 2019	1	1	1	1	2	0	1	1	8
Colombel, 2004	1	1	1	1	0	0	1	0	5
d'Haens, 2017	0	1	1	1	2	0	1	0	6
Ducharme, 2010	0	1	1	1	0	0	1	1	5
Fidder, 2009	1	1	1	1	2	0	1	1	8
Gonci, 2017	1	1	1	1	0	0	1	0	5
Greener, 2018	1	1	1	1	2	0	1	1	8
Kiss, 2013	0	1	1	1	0	0	1	1	5
Lie, 2017	1	1	1	1	2	0	1	1	8
Mourad, 2015	0	1	1	1	0	0	1	1	5
Sartini, 2019	1	1	1	1	0	0	1	0	5
Seiderer, 2004	0	1	1	1	0	0	1	1	5
Teriaky, 2014	1	1	1	1	0	0	1	0	5
Zelinkova, 2012	1	1	1	1	0	0	1	0	5

Table S8.2b Risk of bias assessments. Post-hoc analyses of randomized trials scored using the Cochrane risk of bias assessment tool. Sorted by outcome (efficacy studies, then adverse event studies) and alphabetically by author name.

Author, year	Selection bias	Performance bias	Detection bias	Attrition bias	Reporting bias	Overall judgement
Efficacy studies						
Bouguen, 2015	Possible risk	Low risk	Low risk	Low risk	Possible risk	Unclear risk of bias
De Cruz, 2015	Possible risk	High risk	Low risk	Low risk	Possible risk	Unclear risk of bias
Watanabe, 2018	Possible risk	High risk	High risk	Low risk	Low risk	Unclear risk of bias
Adverse event studies						
Colombel, 2017	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk of bias
Colombel, 2018	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk of bias
Feagan, 2018	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk of bias

Summary and conclusions

Summary and conclusions

Though our understanding of the IBD has massively improved in the last two decades, IBD remains an incurable and therefore chronic disease, often requiring similarly long-term drug use to attain quiescent disease. Knowledge of IBD remains incomplete and knowledge gaps persist concerning both basic science (e.g. etiology and pathogenesis) and clinical practice (e.g. modulation of complications of disease, optimal use of drug therapies). In this thesis we attempted to address several of these knowledge gaps, as summarized below.

In Chapter 1 we have confirmed that topical 5-ASA therapy and topical corticosteroid therapy are considered the most effective therapies for ulcerative proctitis[1], as described in the current guidelines. However, the available literature evidence supporting these assertions had not yet been synthesized. Therefore in Chapter 2 we have examined and analysed the efficacy of topical therapy for ulcerative proctitis using a systematic literature review and meta-analysis.

Two important conclusions can be drawn from this Chapter. Firstly, using the data from 23 individual studies we find that topical 5-ASA is significantly superior to placebo for induction and maintenance of clinical remission, with relative risks ratios of 2.39 and 2.80. This fully supports the guideline recommendations that 5-ASA is effective and is the first choice drug therapy for ulcerative proctitis. Secondly, from the literature review we concluded that there is a lack of high quality evidence concerning other therapies than 5-ASA for ulcerative proctitis. Specifically, we find limited to no evidence for the use of corticosteroids, thiopurines or anti-TNF-agents. Thus, at the time of our search it is only empirical evidence that suggests that topical corticosteroids are effective in the treatment of ulcerative proctitis. However, since publication of our review, several new studies have provided evidence supporting the use of topical steroids for ulcerative proctitis[2-3].

Chapter 3 we attempt to broaden the therapeutic arsenal of topical therapies for ulcerative proctitis. In a randomized controlled trial, we investigate the use of topical tacrolimus for the induction of remission in 5-ASA refractory ulcerative proctitis. We chose to use tacrolimus based on several small studies which showed promising results[4] and decided to use the corticosteroid beclomethasone as an active comparator, as opposed to previous studies which employed a placebo[5]. In total 85 patients were randomized between tacrolimus suppositories and beclomethasone suppositories. After 4 weeks of treatment, the primary outcome of clinical response was seen in 63%

of tacrolimus treated patients and in 59% of beclomethasone patients, a statistically non-significant difference. Similarly there were no statistically significant differences between secondary outcomes such as endoscopic outcomes.

The results of Chapter 3 were unexpected as our working hypotheses consisted of tacrolimus being superior to beclometasone, based on the efficacy observed in previous studies. Thus from this Chapter we can only conclude that tacrolimus suppositories are not superior to beclomethasone suppositories for the treatment of 5-ASA refractory ulcerative proctitis. The results suggest a more or less similar efficacy, and thus the use of either agent seems suitable in the case of 5-ASA refractory disease.

As no cure yet exists for IBD, there is a continuous search for therapies that may influence the chronic gut inflammation. Classical drug research and development, as seen in pharmaceutical companies, is a time and resource intensive process, often taking at least 10 years[6] and costing hundreds of millions (and occasionally more than one billion) of dollars[7]. Therefore if an existing drug also happens to show immunomodulatory effects, it seems sensible to assess its efficacy in a real world setting.

In Chapter 4 we assessed the effects of the drug naltrexone, originally designed for opioid withdrawal, in cellular models and in IBD patients. We find that in the in vitro experiments, exposure to naltrexone at low doses reduced endoplasmic reticulum stress and improved wound healing. However, no effect was seen regarding cytokine levels, neither in cell cultures or in patient material. When applied in a real world population of therapy refractory IBD patients, the effects of low dose naltrexone were promising with short term clinical improvement seen in almost 75% of patients.

When interpreting the results of Chapter 4, we must keep in mind that the patients in the study cohort consist of a highly selected group of patients. Therefore it is difficult to extrapolate the results of this Chapter to the general IBD population. Additionally, IBD is known for its relapsing and remitting behaviour. Thus in our limited time of follow-up, it is difficult to discern if the clinical improvement was the result of naltrexone or the natural course of the disease. The results of this Chapter warrant additional research of a higher quality, such as a randomized controlled trial. In fact, preparations for such a trial are currently underway in the Erasmus MC[8].

As apparent from the name, in IBD the symptoms that patients experience are mostly related to the gut inflammation. However, extra-intestinal symptoms occur frequently[9-

[10]in IBD patients. Though many of these symptoms improve when gut inflammation is treated successfully, debilitating fatigue may remain despite achieving mucosal healing. In Chapter 5 we assess the issue of fatigue in IBD patients. After reviewing the existing literature, we concluded that there is no standardized method of assessing and addressing fatigue in IBD patients. Therefore we proposed a management strategy centred around a fatigue attention cycle. The cycle consists of 7 steps and provides caregivers with a evidence based method to diagnose fatigue, treat possible underlying or associated conditions and re-evaluate the effects of treatment. If no underlying issues are present, non-pharmacological interventions such as lifestyle interventions[11] or cognitive behavioural therapy[12] appear most useful. However besides these two interventions, we could find no evidence for other therapeutic options to treat debilitating fatigue.

To optimize the administration of drugs, understanding of the pharmacodynamics and pharmacokinetics of a drug is essential. With sufficient understanding of these parameters, drug exposure can be manipulated through changes in dose per administration or the interval between doses. In Chapter 6 we perform pharmacokinetic analyses of the drug adalimumab, based on drug level measurements in a large cohort of Crohn's disease patients. We find that a steady state is achieved after 12 weeks of treatment, with mean levels in the cohort of 9.5µg/mL. An assessment of the influence of patient factors on the serum level did not reveal clinically significant influences of factors such as body mass index or patient sex. The most interesting find of this Chapter concerned the steady state of adalimumab. At the steady state, even when doses were (inadvertedly) adminstered later than intended, the measured drug levels remained stable. This implies that in some patients a steady state could be maintained with longer dosing intervals than currently employed. This in turn would lead to reduced drug usage and thus lowered costs, drug administration associated adverse events, whilst not compromising efficacy. However what the actual effects of dose interval lengthening would be requires a completely different study, which is currently underway[13].

Though randomized controlled drug studies are considered one of the highest level of evidence[14], these type of studies are often limited by their rigidly selected patient populations. As such, cohorts of real-life patients can provide different valuable insights into the real world efficacy, safety and tolerability of drug therapies. In Chapter 7 we study a real-life cohort of Crohn's disease patients using adalimumab and assessed safety and tolerability. In particular we focused on possible differences between male and female patients, and noticed several sex differences in outcomes. Most strikingly,

female patients stopped their adalimumab treatment more often than male patients (69% versus 52%), and female patients stopped more often due to side-effects than male patients (35% versus 18%). Thus there appears to be a sex difference in the persistent use and tolerability of adalimumab therapy. Unfortunately, the mechanism behind these findings remains unclear. Potential causes are sex differences in drug metabolism, due to differences in cytochrome activity[15]or effects of sex hormones[16]. Of note, the findings of this Chapter have recently been reproduced in another Dutch cohort[17].

In Chapter 8, we further investigated the possible sex differences in IBD patients treated with biological therapies. Using a systematic literature review and meta-analysis, we eventually identify 55 studies of interest. When assessing endoscopic efficacy of biological agents, we found no evidence for a sex difference in the literature. However, when examining adverse events, 7 out of 14 relevant studies reported an increased risk of adverse events occurring in female patients. This suggests that the sex difference could be partly explained by female patients being more prone to adverse events in general. This concept of increased adverse event rates in female (non-IBD) patients is also reflected in the literature[18-19]. The results of this Chapter suggest that in clinical practice it remains essential to pro-actively discuss potential side effects with patients. Furthermore, clinicians should continuously assess for the presence of side effects in during drug treatment.

Future recommendations and concluding remarks

IBD remains a field in motion with many unknowns, though new discoveries are frequently made concerning etiology and pathogenesis, with subsequent development of new treatments. This thesis has attempted to fill in some of the existing knowledge gaps, via randomized trials, systematic literature reviews, meta-analyses and cohort studies. Nevertheless, the new insights gained in this thesis have also exposed interesting new gaps in our knowledge.

Currently there are several projects ongoing or in preparation that address these newly identified knowledge gaps. For instance, partly inspired by the results of Chapter 6, adalimumab dose interval lengthening is currently being studied in several Dutch hospitals in the 'Lengthening Adalimumab Dosing Interval' (LADI) study[13]. Similarly, based on the findings concerning naltrexone as described in Chapter 4, a randomized

controlled trial comparing low dose naltrexone with azathioprine is currently being prepared[18].

Another potential avenue for for further research is the potential sex differences in biological therapy related adverse events. Perhaps when utilizing adverse event data collected during randomized controlled trials (in post-hoc fashion), a more robust analysis could be performed to discern between general adverse events during treatment and potentially drug related adverse events.

However, even if all the knowledge gaps identified in this thesis are examined in the future, undoubtedly new gaps will be identified in those studies. And so the perennial cycle of medical research will continue.

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Nederlandse samenvatting en conclusies

Nederlandse samenvatting en conclusies

Inleiding

De inflammatoire darmziekten, vaak afgekort als IBD, betreft een groep van chronische ontstekingen aan de darmen. In praktische zin bestaat IBD uit twee ziektebeelden, namelijk de ziekte van Crohn en colitis ulcerosa. Deze ziekten onderscheiden zich door verschil in locatie van de darmontsteking, en verschil in het gedrag van de darmontsteking.

De exacte oorzaak van IBD is tot op heden onduidelijk, hoewel in de afgelopen decennia onze kennis van IBD aanzienlijk is toegenomen. Met de kennis van nu ligt de oorzaak van IBD waarschijnlijk in een combinatie van factoren, zoals genetische aanleg[1-2], omgevingsfactoren[3-4] en een verstoorde reactie van het afweersysteem op de bacteriën in de darm[5].

Behalve een toename in kennis, is er de laatste decennia ook een wereldwijde toename in het aantal patiënten met IBD[6]. Intussen zijn er ten minste 80.000 IBD patiënten in Nederland, en de verwachting is dat dit aantal alleen zal toenemen.

Aangezien IBD een chronische ziekte is, is in veel gevallen ook een chronische ('levenslange') behandeling noodzakelijk. Aangezien IBD vaak op jonge leeftijd wordt vastgesteld[7] gaat de chronische behandeling van IBD gepaard met hoge zorgkosten[8]. Daarnaast kan IBD, met name als de ziekte niet goed wordt behandeld, leiden tot verminderde kwaliteit van leven[9-11] en verminderde productiviteit[12-13], waardoor de maatschappelijke kosten nog hoger worden.

Het is dus zowel vanuit oogpunt van de patiënt, als vanuit oogpunt van de maatschappij, van groot belang om de ziekte zo goed mogelijk onder controle te krijgen. Helaas is tot op heden genezing van IBD niet mogelijk, ondanks de toename in kennis over het ziektebeeld. Het beste wat men nu kan nastreven is verlichting van symptomen met volledige onderdrukking van de ontsteking in de darmen. Het onderdrukken van de ontsteking is belangrijk, omdat daarmee complicaties van de darmontsteking (zoals littekenvorming in de darm) voorkomen wordt[14].

Bij elke behandeling van een ziekte moet de balans gezocht worden tussen effectiviteit van een medicijn, en de bijwerkingen van een medicijn. Vaak zullen sterkere medicamenten meer of ernstigere bijwerkingen hebben dan relatief zwakke

medicijnen. Binnen de behandeling van IBD geldt eenzelfde onderverdeling, waarbij er relatief zwakke en relatief sterke behandelingen bestaan. De richtlijnen voor de behandeling van IBD[15-16] adviseren een stappenplan, waarbij in de meeste patiënten gestart wordt met de zwakste medicamenten. Indien de zwakste medicamenten onvoldoende werken of niet worden verdragen, kan een zwaarder middel ingezet worden totdat de ontsteking in de darmen onder controle is.

Samenvatting van het proefschrift

Ondanks de toename in kennis omtrent het ontstaan van IBD en de ontwikkeling van nieuwe behandelingen, bestaan er nog volop onbeantwoorde vragen omtrent IBD. IBD blijft tot op heden een ziekte die we niet kunnen genezen, met navenante noodzaak tot chronische behandeling, met de bijbehorende persoonlijke en maatschappelijke consequenties. Met dit proefschrift hebben we gepoogd een aantal van de kennishiaten binnen de IBD op te vullen.

Bij IBD-patiënten met colitis ulcerosa bevindt de darmontsteking zich in de dikke darm. Bij een deel van deze patiënten is de ontsteking beperkt tot de endeldarm, dit type ontsteking wordt proctitis ulcerosa genoemd. Bij patiënten met proctitis ulcerosa wordt lokale behandeling met in eerste instantie 5-ASA preparaten en in tweede instantie corticosteroïden gezien als de juiste volgorde van behandeling[15]. Dit is zo beschreven in de richtlijnen, zoals we resumeren in Hoofdstuk 1. Echter, ondanks deze duidelijke aanbevelingen in de richtlijn, schort het aan een goede analyse van al het beschikbare bewijs uit de wetenschappelijke literatuur. In Hoofdstuk 2 hebben we door middel van systematisch literatuuronderzoek de beschikbare bewijslast geïdentificeerd. Vervolgens hebben we door middel van meta-analyse gegevens uit 23 verschillende studies geaggregeerd, waaruit inderdaad blijkt dat lokale behandeling met 5-ASA preparaten duidelijk effectiever is dan een behandeling met placebo. De *relative risk ratio's* voor het induceren en onderhouden van ziekte remissie zijn respectievelijk 2.39 en 2.80 voor 5-ASA vergeleken met placebo. Wat echter ook uit deze literatuurstudie blijkt, is dat de bewijslast voor het gebruik van corticosteroïden veel beperkter is. Echter, sinds publicatie van Hoofdstuk 2 zijn resultaten van nieuwe studies bekend geworden, waarin de waarde van lokale corticosteroïden wel wordt bewezen[17-18].

In Hoofdstuk 3 trachten we door middel van een gerandomiseerde studie meer behandelopties te identificeren voor patiënten met actieve proctitis ulcerosa. In dit onderzoek hebben we besloten om het middel tacrolimus te onderzoeken, vanwege de veelbelovende resultaten die dit middel heeft getoond in eerdere onderzoeken[19].

Aangezien het onderzoek patiënten betrof met actieve ontsteking ondanks behandeling met 5-ASA (de eerste keus behandeling), hebben we in dit onderzoek de helft van de patiënten behandeld met tacrolimus, en de andere helft met beclometason (een corticosteroïd, de tweede keus behandeling). Uiteindelijk hebben we 85 patiënten behandeld met tacrolimus of beclometason zetpillen. Na 4 weken behandeling waren de klachten en de darmontsteking bij de meeste patiënten afgenomen of verdwenen. Het effect van tacrolimus was echter hetzelfde als het effect van beclometason, er werd namelijk een positief effect gezien in respectievelijk 63% en 59% van de patiënten. Deze uitkomst was enigszins onverwacht, aangezien we op grond van de eerdere onderzoeken hadden verwacht dat tacrolimus effectiever zou zijn dan beclometason. Gezien de opbouw van de studie kunnen we met deze resultaten dan ook alleen concluderen dat het gebruik van tacrolimus niet beter is dan het gebruik van beclometason. Waarschijnlijk zijn beide middelen dus geschikt om patiënten te behandelen met actieve proctitis ulcerosa als 5-ASA onvoldoende heeft gewerkt.

Zoals eerder gezegd is er tot op heden is er nog geen genezing mogelijk voor IBD. Voor veel patiënten is een chronische behandeling met medicatie dan ook nodig om de ontsteking van de darm te onderdrukken. Het ontwikkelen van medicatie is een kostbare en tijdrovende zaak, geschat wordt dat de ontwikkelingskosten van een medicament tot wel 1 miljard dollar kunnen oplopen[20] over een periode van 10 tot 15 jaar[21]. Soms blijken echter bestaande medicijnen een onverwachte werking te hebben op gebieden waarvoor het niet origineel is ontwikkeld. Een voorbeeld hiervan is het middel naltrexon, wat origineel ontwikkeld is voor ontwenningsverschijnselen. Naltrexon blijkt echter ook ontstekingsactiviteit te kunnen verminderen, waarbij er aanwijzingen zijn dat dit ook geldt voor ontsteking in de darm.

In Hoofdstuk 4 beschrijven we dan ook de inzichten die we hebben opgedaan met naltrexon, zowel in laboratoriumonderzoeken alsmede in daadwerkelijke IBD patiënten. Uit de laboratoriumonderzoeken blijkt dat naltrexon een positieve invloed heeft op wondgenezing. Tevens neemt de *endoplasmische reticulum stress* af, wat mogelijk een positieve invloed heeft op ontsteking en cel verval. Deze effecten blijken los te staan van niveaus van cytokines die een rol spelen in de bekende ontstekingsprocessen binnen IBD. De bevindingen uit de laboratoriumonderzoeken lijken we ook terug te zien in de patiënten die zijn behandeld met naltrexon. Zo zien we dat tot wel 75% van de patiënten op de korte termijn een afname van klachten meldt na de behandeling met naltrexon. Echter, dit was geen gestructureerd patiënten onderzoek, waardoor er niet met hoge zekerheid geconcludeerd kan worden dat naltrexon een daadwerkelijk effectieve of geschikte behandeling is. Dit temeer omdat bij IBD bekend is dat er binnen

korte tijd de symptomen erg kunnen schommelen. In elk geval kan uit dit hoofdstuk wel geconcludeerd worden dat de effecten van naltrexon veelbelovend zijn, en inmiddels worden voorbereiding getroffen voor een goed gestructureerd en prospectief onderzoek[22].

Zoals de naam van het ziektebeeld al impliceert, is ontsteking van de darm het hoofdprobleem bij IBD. De meeste klachten die patiënten ervaren zijn dan ook direct gerelateerd aan de ontsteking van de darm. Een aanzienlijk deel van de patiënten heeft echter ook symptomen die niet direct gerelateerd zijn aan de darmontsteking, zogenaamde extra-intestinale symptomen[23-24]. Hoewel veel van deze extra-intestinale klachten afnemen als de darmontsteking ook afneemt, kunnen sommige klachten blijven bestaan ondanks een volledig rustige darm. Een van deze vaak persisterende symptomen is ernstige vermoeidheid. In Hoofdstuk 5 verdiepen we ons in deze ernstige vermoeidheid. In eerste instantie hebben we de bestaande literatuur bestudeerd, waaruit blijkt dat er geen goede gestandaardiseerde methoden of adviezen bestaan voor de aanpak van vermoeidheid bij IBD patiënten. Op grond hiervan hebben we dan ook een voorstel gedaan om vermoeidheid structureel te analyseren en behandelen, door middel van een stappenplan. Het aanpakken van direct behandelbare cofactoren, zoals ontsteking aan de darm of stress, staat hierbij voorop. Desondanks kan vermoeidheid blijven bestaan, in dergelijke gevallen kunnen lifestyle interventies[25] en cognitieve gedragstherapie[26] nog een verbetering geven. Desondanks leert de ervaring dat een deel van de patiënten last blijft houden van ernstige en moeilijk te behandelen vermoeidheid.

Bij het toedienen van medicatie dient altijd een afweging te worden gemaakt tussen de effectiviteit en toxiciteit. Hoewel meer medicatie kan leiden tot een sterker effect, neemt bij een hogere dosis de kans op bijwerkingen veelal ook toe. Hierin ligt dan ook de waarde van onderzoek naar optimalisatie van medicatietoedieningen. In Hoofdstuk 6 hebben we onderzoek verricht naar de farmacokinetiek van het middel adalimumab. Door middel van het meten van medicatiespiegels van het middel adalimumab in het bloed van 76 patiënten we vastgesteld dat er een stabiele situatie ontstaat na circa 12 weken behandeling. Opvallend is dat patiëntgebonden factoren, zoals het gewicht of geslacht van de patiënt, weinig invloed hebben op de medicatiespiegel. Een nog interessantere bevinding betreft de stabiliteit van de spiegel. Normaliter wordt adalimumab elke 2 weken toegediend, maar bij patiënten die (per ongeluk) het pas na 3 weken toedienden bleef de spiegel toch stabiel. Mogelijk kan er dus met minder frequente toediening toch een stabiele spiegel worden gehandhaafd, waarbij de effectiviteit mogelijk hetzelfde blijft terwijl de kans op bijwerkingen afneemt. Of het

minder frequent toedienen van medicatie geen risico's met zich mee brengt valt uit dit onderzoek helaas niet op te maken, maar momenteel is een onderzoek gaande wat wellicht antwoordt zal geven op deze vraag[27].

Veelal worden de resultaten van goed opgezette gerandomiseerde studies gezien als de resultaten met een hoge kwaliteit[28]. Helaas zijn dergelijke onderzoeken kostbaar en door de rigide methodologie zijn de resultaten niet altijd volledig toepasbaar op de gangbare patiëntenpopulatie. Cohortonderzoeken in ongeselecteerde patiënten-populaties kunnen dan ook aanvullende inzichten opleveren. In Hoofdstuk 7 beschrijven we een cohort van patiënten met de ziekte van Crohn die behandeld worden met adalimumab. Gedurende de onderzoeksperiode zien we een aantal opvallende verschillen tussen mannelijke en vrouwelijke patiënten. Vrouwelijke patiënten stoppen veel vaker met adalimumab therapie dan mannelijke patiënten (69% versus 52%). Daarbij zien we ook dat vrouwelijke patiënte veel vaker vanwege bijwerkingen hun behandeling stoppen (35% versus 18%). In het cohort wat wij hebben bestudeerd is er dus duidelijk sprake van een sekse verschil, en recentelijk is in een ander Nederlands cohort eenzelfde resultaat vastgesteld[29]. Echter, de reden voor het sekse verschil blijft onduidelijk. Uit de algemene medische literatuur wordt in elk geval duidelijk dat vrouwelijke patiënten over het algemeen vaker bijwerkingen ervaren dan mannen. In sommige gevallen spelen verschillen in het metabolisme van medicatie en rol[30-31] maar dat lijkt niet het geval te zijn bij adalimumab therapie.

In Hoofdstuk 8 verdiepen we ons verder in de mogelijke sekse verschillen bij IBD patiënten die behandeld worden met biologicals. Via een uitgebreid en systematisch literatuuronderzoek hebben we 55 studies geïdentificeerd die in enige mate het mogelijke sekse verschil hebben onderzocht. In deze studies komt geen sekse verschil naar voren met betrekking tot effectiviteit van de behandeling. Echter in een deel van de onderzoeken wordt wel een sekse verschil gezien op het gebied van bijwerkingen, waarbij er bij vrouwelijke patiënten meer bijwerkingen worden vastgesteld dan bij mannelijke patiënten. Hierbij dient wel vermeld te worden dat de een deel van deze onderzoeken geen onderscheid heeft gemaakt tussen algemene bijwerkingen (zoals hoofdpijn of misselijkheid) en bijwerkingen die potentieel gerelateerd zijn aan de medicatie. Daarnaast waren de resultaten van de verschillende studies niet geschikt voor aggregatie via meta-analyse. Desondanks laat dit onderzoek wel aanwijzingen zien voor een mogelijk sekse verschil op het gebied van bijwerkingen. De vraag blijft echter wel of deze bevinding alleen geldt voor IBD patiënten die behandeld worden met biologicals, of dat deze bevinding op een bredere populatie van toepassing is.

Conclusies en toekomstperspectief

IBD blijft een fascinerend onderzoeksgebied met frequente nieuwe ontwikkelingen en ontdekkingen. Hoewel er op deze wijze steeds nieuwe inzichten worden vergaard, leiden nieuwe inzichten vrijwel altijd ook tot nieuwe vragen. In dit proefschrift hebben we getracht door middel van de uitgevoerde studies een aantal van deze kennishiaten op te vullen. Hoewel we daar deels in zijn geslaagd, roepen de studies beschreven in dit proefschrift ook weer nieuwe vragen op.

Momenteel zijn er enkele projecten gaande of in voorbereiding die deze nieuwe kennishiaten kunnen opvullen. Zo is momenteel de 'Lengthening Adalimumab Dosing Interval' (LADI) studie gaande[27], deels op basis van de resultaten van het onderzoek uit Hoofdstuk 6. Op vergelijkbare wijze zijn de gegevens omtrent naltrexon uit Hoofdstuk 4 de basis van een aankomend onderzoek waarin behandeling met naltrexon wordt vergeleken met behandeling met azathioprine[22].

Andere interessante onderzoeksgebieden zijn de mogelijke man-vrouw verschillen betreffende bijwerkingen van biologicals. De resultaten van Hoofdstuk 8 geven helaas geen eenduidig beeld over het mogelijke man-vrouw verschil, maar wellicht dat deze vraag alsnog beantwoord zou kunnen worden door middel van een post-hoc analyse, gebruikmakend van de bijwerkingen registraties van de eerste grote gerandomiseerde onderzoeken naar biologicals.

Echter, zelfs al worden alle huidige kennishiaten opgevuld dankzij nieuw onderzoek, het is vrijwel zeker dat er op basis van die onderzoeken weer nieuwe hiaten worden blootgelegd. En dat beschrijft precies de eindeloze cirkel van het medisch wetenschappelijk onderzoek.

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Appendices

Abbreviations

Bibliography

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Appendices

Abbreviations

5-ASA	5-aminosalicylic acid
6-MP	6-mercaptopurine
6-TG	6-tioguanine
ADA	adalimumab
AZA	azathioprine
BID	*bis in die*, two times daily
BMI	body mass index
CD	Crohn's disease
CDAI	Crohn's disease activity index
CFS	chronic fatigue syndrome
CI	confidence interval
CIS	Checklist Individual Strength
CYP450	cytochrome P450
CRP	C-reactive protein
eGFR	estimated glomerular filtration rate
ECCO	European Crohn's and Colitis Organisation
EOW	every other week
GPT	N-acetylglucosamine-1-phosphate transferase
HR	hazard ratio
IBD	inflammatory bowel disease
IBDQ	inflammatory bowel disease questionnaire
IEC	intestinal epithelial cells
IFX	infliximab
ELISA	enzyme linked immunosorbent assay
IQR	interquartile range
K-S test	Kolmogorov-Smirnov's test
LDN	low dose naltrexone
LPS	lipopolysaccharide
LUC	left sided ulcerative colitis
MFI-20	Multidimensional Fatigue Inventory
MMF	mycophenolate mofetil
MOR	mu-opioid receptor
MS	multiple sclerosis
MTX	methotrexate
NOS	Newcastle-Ottawa Scale
OD	once daily

OR	odds ratio
PSQI	Pittsburgh Sleep Quality Index
Q-Q plot	quartile-quartile plot
QOL	quality of life
RCT	randomized controlled trial
RR	relative risk
rt-PCR	reverse transcriptase polymerase chain reaction
SASP	sulfasalazine
SC	subcutaneously
SD	standard deviation
SFT	solution-focused therapy
TID	*ter in die*, three times daily
TLR4	Toll like receptor 4
TNF-alpha	tumor necrosis factor alpha
UC	ulcerative colitis
UP	ulcerative proctitis
VAS	visual analog scale

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