SAFETY AND EFFICACY OF INFLAMMATORY BOWEL DISEASE TREATMENT

A CHALLENGING BALANCE

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COLOPHON

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een uitdagende balans

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CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

GENERAL INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory condition affecting the gastro-intestinal tract and encompasses Crohn's disease (CD), ulcerative colitis (UC) and IBD-unclassified (IBDU). Even though these subtypes are considered separate entities, all are characterized by relapsing chronic inflammation with periods of active disease (flares) alternated with quiescent periods (remission). Over the past decades, the incidence and prevalence of IBD have increased worldwide, putting significant burden on both individuals and the health care system. IBD prevalence is highest in developed countries and currently estimated at 0.3% in Europe.[1] Disease onset is typically in early adulthood, and most patients are diagnosed between 20-40 years of age. Although the exact aetiology of IBD remains unclear, current evidence indicates that it involves a complex interplay between the immune system, the gut microbiome, genetic susceptibility and environmental factors. The current hypothesis is that a genetically susceptible person (host) experiences an environmental trigger that causes an inappropriate and perpetuating immune response towards the intestinal microbiota, resulting in chronic immune activation and intestinal inflammation.[2,3]

Disease characteristics

IBD is diagnosed based on a combination of clinical, endoscopic, radiological and histological findings.[4] UC was the first subtype of IBD to be characterized as a distinct entity and is characterized by inflammation extending from the rectum and limited to the colon. Inflammation in UC is continuous and typically involves the superficial mucosal and submucosal layers. Depending on the extent of inflammation in the colon, patients can be classified as having proctitis (inflammation limited to rectum), left-sided colitis (inflammation up to the splenic flexure), or extensive colitis (beyond the splenic flexure).[5] In CD, inflammation can be present along the entire gastrointestinal tract from the oral cavity to the anal region, but the terminal ileum and colon are most commonly affected.[6,7] Inflammation in CD is characterised by a patchy distribution and begins with small superficial aphtous ulcers that can develop into larger transverse linear ulcers.[8-11] The inflammation involves all layers of the bowel wall (transmural). [12,13] In some cases it is impossible to distinguish between CD and UC and these patients are labelled as IBDU.[14-16] When features of the colectomy specimen are insufficient to allow a definitive diagnosis, patients are labelled as IBD-indeterminate (IBDI).[17]

Symptoms and disease manifestations

Patients suffer from a variety of gastro-intestinal symptoms including abdominal pain, diarrhea, bloody stools and urgency. In addition, systemic symptoms like anemia, anorexia and weight loss are common. Most patients suffer from fatigue, especially during active disease periods, but also when disease seems inactive. A significant proportion of patients experience extra-intestinal manifestations like arthralgia, arthritis, uveitis, primary sclerosing cholangitis, and skin abnormalities such as erythema nodosum, hidradenitis suppurativa and pyoderma

gangrenosum. Both intestinal and extra-intestinal symptoms negatively affect the physical well-being and quality of life of IBD patients.[18-20]

Natural history

The course of IBD is difficult to predict because disease onset, severity and duration of flares vary widely among patients. IBD is usually characterized by alternating episodes of flares and remission and can follow various patterns, from aggressive to indolent types.[21-23] The majority of patients present with moderate-to-severe symptoms at diagnosis, but disease activity tends to decrease over time.[24] Nearly all patients have a disease flare after the initial clinical episode and over 50% experience a relapse within the first year after diagnosis.[25,26] Recurrent flares or untreated inflammation are associated with irreversible damage to the gastro-intestinal tract such as bowel perforation or formation of strictures, abscesses and fistulas. CD patients in particular are prone to develop these disease complications because of transmural inflammation.[12,13] Fistulas are inflammatory tracts that form a connection between the intestine and the skin, bladder, vagina or other bowel segments. Disease complications develop in 70-75% of CD patients and are associated with significant morbidity and often require hospitalization and surgery.[12,13,27-31] These disease complications are rare in UC because of superficial bowel inflammation. However, data suggest that a 10-15% of UC patients experience disease progression to extensive colitis and some patients do require surgery (colectomy).[25,32] More importantly, IBD patients are at increased risk for colorectal cancer, a result of chronic (histological) inflammation in the colon, causing continuous regeneration of bowel cells.[33]

MEDICAL TREATMENT

Since IBD is a complex, multifactorial disease with a progressive natural history and invalidating symptoms, and a 'cure' has not been identified, most patients require long-term medical therapy with anti-inflammatory drugs or surgery to treat disease flares and complications and to reduce the risk of a relapse. Over the years several new anti-inflammatory drugs targeting different aspects of the immune system, have become available. Classification and positioning of these different IBD drugs rely on their properties; induction therapies are characterised by a rapid onset of action to induce response and remission, and maintenance therapies to maintain remission are appropriate for long-term use. Some drugs are fit both as induction and maintenance therapies.

Corticosteroids were amongst the first drugs used in the treatment of IBD and have proven to be effective in induction of disease remission.[34,35] These drugs bind to the glucocorticoid receptor and stimulate transcription of anti-inflammatory genes and inactivates pro-inflammatory transcription factors (nuclear factor κβ, activator protein-1) thereby preventing activation of inflammatory mediators (e.g. leukotrienes and cytokines such as IL-1 and IL-6).[36,37]Current treatment strategies limit the use of corticosteroids to induction courses. Moreover, corticosteroid-sparing strategies have been advocated over recent decades since glucocorticosteroids are insufficiently effective as maintenance treatment and associated with

serious side effects, especially in high dosages or long-term use. Side effects include infections, hyperglycemia, psychosis, osteopenia, growth failure and weight gain.[38,39]

Aminosalicylates, mainly 5-aminosalicylic acid (5-ASA) serve both as induction therapy and maintenance treatment and might reduce the risk of colonic cancer.[40] Several mechanisms of action for 5-ASA have been proposed including reduction of prostaglandin synthesis via local inhibition of pro-inflammatory cytokines and oxygen-free radicals, and inhibition of T-cell proliferation and activation.[41,42] It is an effective treatment in the majority of UC patients (90%), particularly in those with mild to moderate ulcerative colitis.[43] 5-ASA has a favourable safety profile and is well tolerated with subsequent high treatment adherence. 5-ASA can be administered orally or rectally (suppository or enema). Local treatment with suppositories or enemas is an appealing treatment option to manage patients with inflammation limited to the rectum and left-sided colon in certain patients. Although frequently prescribed in the past, the efficacy of 5-ASA in CD has been questioned and current guidelines advice against the use of 5-ASA for induction of remission.[44,45] Cases of impaired renal function have been sparsely mentioned usually managed with drug withdrawal.[46]

Immunomodulators comprise purine analogues (azathioprine, mercaptopurine, tioguanine), folate antagonists (methotrexate) and calcineurin inhibitors (ciclosporin, tacrolimus). Purine analogues (i.e. azathioprine (AZA), mercaptopurine (MP) and tioguanine (TG) have been used in medical treatment of IBD with over 50 years of global experience.[47] Thiopurines were considered after therapy failure of aminosalicylates and in steroid-refractory or steroid-dependent IBD patients, but are nowadays applied as a maintenance strategy, and in moderate CD as a primary treatment. Advantages include a steroid-sparing effect, low costs and its association with a reduced risk of colorectal carcinoma.[48,49] A recent study showed that thiopurine continuation in UC was associated with a lower rate of hospital admission and a reduced risk of progression of disease extent and colectomy.[50] Also, in the past decades they have been used in combination treatment alongside a biologic agent with proven synergistic effects and reduction of antidrug-antibody formation (immunogenicity).[51] AZA and MP are pharmacologically inactive pro-drugs, which are converted by three competing enzymatic pathways to produce, amongst others, the pharmacologically active metabolites 6-thio-guanine nucleotides (6-TGN) and 6-methylmercaptopurine ribonucleotides (6-MMPR). TG undergoes direct enzymatic conversion towards the formation of 6-TGN (**Figure 1**).

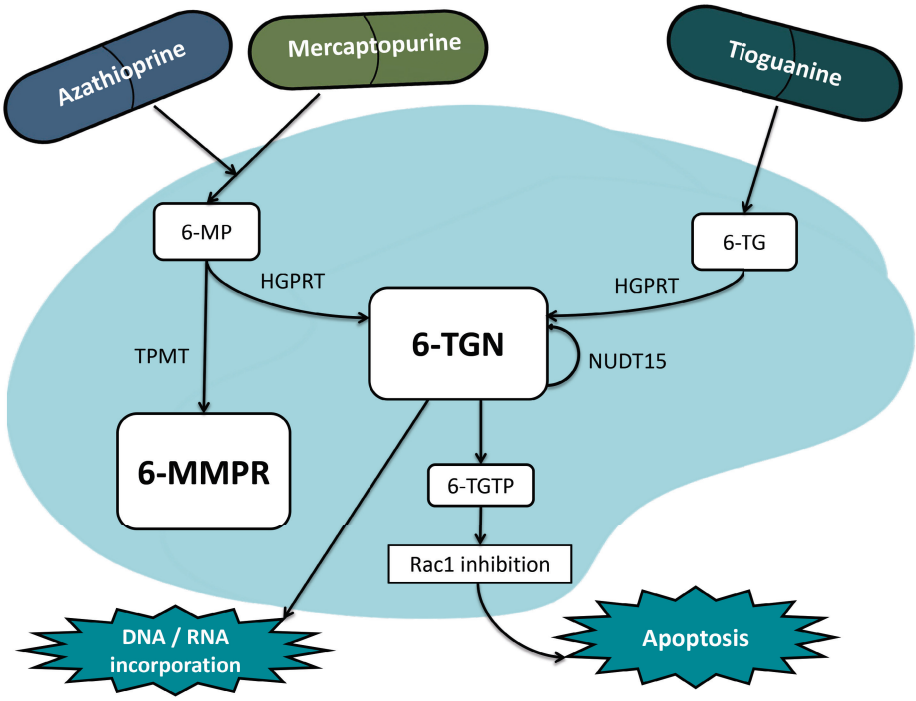

Figure 1. Simplified model of thiopurine metabolism.

6-MP, 6-mercaptopurine; 6-TGN, 6-thioguanine; 6-MMPR, 6-methylmercaptopurine ribonucleotides; 6-TG, 6-thioguanine; HGPRT, hypoxantine-guanine phosphoribosyl transferase; TPMT thiopurine S-methyltransferase; 6-TGTP, 6-thioguanosine 5' triphosphate.

The immunosuppressive effects and predominantly assigned to the metabolite 6-TGN (more specifically 6-thioguanine triphosphate) that inhibits the activity of Rac1, thereby inducing apoptosis of activated T lymphocytes.[52,53] Another route of immunosuppression involves conversion of 6-TGN into deoxy-6-thioguanosine, which causes cytotoxicity by incorporation into DNA and RNA.[54] Clinical effectiveness is correlated with 6-TGN, but high levels are associated with myelotoxicity. Conversely, the metabolites 6-MMPR have been associated with therapy refractory disease and adverse events such as hepatotoxicity.[55-57] The therapeutic window of thiopurines is small and some patients withdraw from treatment because of lack of efficacy or adverse events.[58] Both efficacy and toxicity of thiopurines are dependent on the extent to which 6-TGN and 6-MMPR metabolites are produced and often varies between patients because of large inter-individual variation in thiopurine metabolism.[55-58] Genetic polymorphisms of the thiopurine metabolizing enzymes thiopurine S-methyl transferase (TPMT) and NUDT15 in part explain variable enzyme activity and have been associated with the development of myelotoxicity. Nowadays, genetic testing for TPMT mutations before the start of thiopurine treatment is suggested to avoid (early) myelotoxicity.[59] Another strategy to avoid therapy withdrawal is therapeutic drug monitoring (TDM) using 6-TGN and 6-MMPR metabolite measurements to adjust thiopurine dose in patients with inadequate response or adverse events. Patients with a 'skewed metabolism' produce high levels of the potentially

toxic 6-MMPR, whereas favourable 6-TGN levels remain below therapeutic range and are therefore particularly at risk of thiopurine withdrawal. Two important optimization strategies can be considered in patients on AZA or MP with 6-MMPR associated intolerance or treatment failure. First, the addition of allopurinol, a xanthine oxidase inhibitor, to a reduced thiopurine dose redirects the thiopurine metabolism towards 6-TGN formation, resulting in increased 6-TGN levels and decreased 6-MMPR levels.[60,61] Second, switching treatment to TG bypasses several intermediate metabolites associated with the majority of adverse events.[62-65] Both strategies improve effectiveness and tolerability of thiopurines.[66]

The folate antagonist methotrexate (MTX) affects survival of immune cells by inhibition of cell proliferation, suppression of pro-inflammatory cytokine production and induction of apoptosis.[67,68] MTX has moderate efficacy in CD as a steroid-sparing induction therapy, but mainly as a maintenance therapy. MTX is largely inneffective in UC.[69-71] As with thiopurines, MTX can be used as monotherapy or in combination with biologics to reduce immunogenicity. Up to one third of patients discontinue MTX because of intolerance, and cases of myelotoxicity, hepatotoxicity and interstitial pneumonitis have been described.[72]

Although less frequently prescribed, systemically applied calcineurin inhibitors are an established therapeutic option for steroid-refractory ulcerative colitis.[73] Calcineurin inhibitors bind to specific intracellular receptors and act by blocking the activation of transcription factor nuclear factor of activated T-cells and thereby inhibit cytokine gene transcription and promote T-cell apoptosis.[74,75] Ciclosporine-A has been used as an intravenous rescue therapy for acute severe colitis.[73,76] Tacrolimus was also studied as a local therapy and found to be safe and effective as an induction therapy.[77,78]

The introduction of *biologics* in the late nineties has brought substantial advances in the medical treatment of IBD. The first biologics were directed towards the pro-inflammatory cytokine ‘tumor necrosis factor α’ (i.e. anti-TNF) produced in various cells in the inflamed gut of IBD patients.[79,80] Anti-TNF agents are large protein molecules and consist of solely human (adalimumab, golimumab and certolizumab) or both human and mouse (infliximab) monoclonal antibodies. These drugs have a rapid onset of action and are potent for both inducing remission, maintaining remission and reduce the need for hospitalization and surgery.[81,82] Despite the proven benefit of these therapies, a significant number of patients (30%) will not respond to anti-TNF or fails to maintain response (40%).[83-87] The development of anti-drug antibodies with neutralizing properties that reduce the efficacy of the drug (immunogenicity), play an important role in treatment failure.[88] Concomitant use of immunomodulators has a beneficial effect, both synergistic in effectiveness as well as in decreasing the risk of immunogenicity.[89] Years later, new groups of biologics have entered the market such as selective anti-integrins (vedolizumab, natalizumab) some with gut-selective anti-inflammatory effects, and anti-interleukins (ustekinumab). Another drug group that has been explored concerns new small molecules including Janus kinase inhibitors (tofacitinib).[90-93] At the time of writing this thesis, a plethora of new drugs are being tested in clinical trials

in IBD. Many of these new therapeutic approaches have been developed on the basis of studies in IBD mouse models, genetic studies, analyses of IBD tissues and new insights into inflammatory pathways in other chronic inflammatory disorders, such as rheumatoid arthritis and psoriasis.[94-96]

THERAPEUTIC APPROACH

The therapeutic approach in IBD management has evolved considerably over the years since treatment goals have shifted, and are facilitated by the increasing number of available therapies. In the past, treatment in IBD focussed primarily on symptom relief induction and maintenance of clinical remission and ultimately corticosteroid-sparing management and avoiding surgery. Patients were treated following a "*step-up*" approach where the different medication classes are successively introduced after failure of a previous drug, starting with the least aggressive therapy (**Figure 2**). [97]Therapeutic approaches driven only by symptomatic control of disease activity failed to change the natural disease course.[98,99] Therefore, the therapeutic paradigm changed to an *'accelerated step-up'* approach' in which more aggressive medication is initiated early in the treatment combined with more stringent assessment of disease activity and rapid therapeutic escalation to achieve tight control of inflammation and prevent "bowel damage". [100,101] Current treatment goals comprise clinical and steroid-free remission, mucosal healing, and prevention of hospital admission, surgery, inflammatory-related malignancy and improvement of quality of life.[100-108] Another strategy is the *'top-down'* approach in which biologics are introduced early in the disease course, usually combined with an immunomodulator (**Figure 2**).

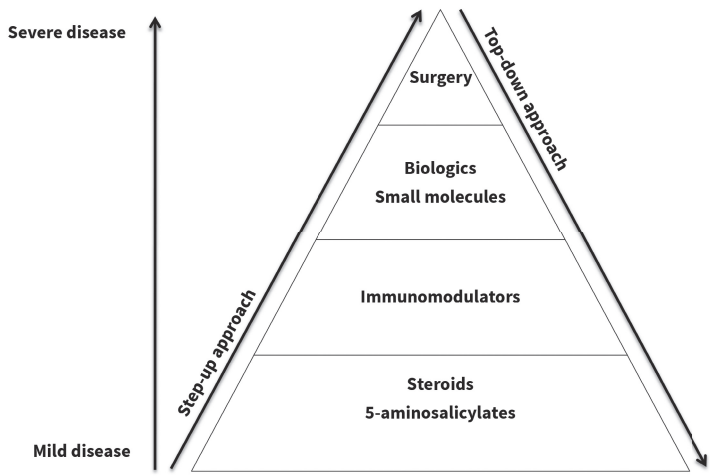

Figure 2. IBD treatment pyramid

These strategies are mostly applied in patients presenting with severe disease characteristics, disadvantageous prognostic factors or early signs of therapeutic failure. It has been shown that the top-down approach was more effective for induction of remission than the

conventional step-up approach.[109] The precise efficacy of the top-down approach in the long term is not completely clear, although some studies report reduced complications and surgical rates.[110,111]

RISKS IN MEDICAL TREATMENT

IBD is a chronic disease requiring long-term medical treatment in most patients. Although these therapies have a clear benefit in IBD, certain treatment-associated risks need to be considered when choosing a therapeutic strategy. While disease-related morbidity is feared in step-up strategies, the risk of overtreatment with top-down strategies is almost inevitable. Patients are exposed to drug-specific adverse events as discussed above but the risk of infections and certain types of cancer is also increased. On the one hand, treatment of IBD aims for mucosal healing and deep remission, thereby decreasing the risk of intestinal infections related to intestinal lesions and reducing the risk of cancers associated with chronic mucosal inflammation. On the other hand, immunosuppressive treatments have been shown to promote (opportunistic) infections and certain types of cancer in IBD patients.[112] The nature and magnitude of the increased risk of infections and cancer vary with patient sex, age and immunosuppressive drug class.

Infections

The severity of infections varies among patients and can present as mild infections, serious infections that require hospitalization and life-threatening opportunistic infections that only occur in immune-compromised patients.[113-115] The majority are mild viral infections, usually related to immunomodulator exposure, but cases of fatal infections in young patients have been described.[116,117] There is an excess risk of all types of infections in patients exposed to corticosteroids, immunomodulators and/or anti-TNF agents, particularly older patients.[114,118] The risk of opportunistic infections is highest in the setting of combination therapy with immunomodulators and anti-TNF agents, or exposure to corticosteroids.[114,119,120] Myelotoxicity, a known adverse event of thiopurine therap, further increases the risk of opportunistic infections.[113,121] To timely detect myelotoxicity, frequent monitoring of the full blood count (FBC) is performed throughout therapy with thiopurines or methotrexate. Also, patients are usually vaccinated before initiation of immunomodulators or biologics for viruses like hepatitis B, and also screened for latent infections that can reactivate in the setting of immunosuppressive treatment such as tuberculosis.

Cancer

IBD patients are 2–6 times more likely to develop colorectal cancer (CRC) than the general population.[122] The risk of CRC is higher in UC than in CD patients, and increases with disease duration.[123,124] Persistent histological inflammation is also a prerequisite for the development of CRC and therefore the risk is particularly high in patients with chronic active disease.[125,126] In addition, IBD patients are at increased risk of extra-intestinal cancers including skin cancer and lymphoproliferative disorders and cervical neoplasia.[14,127,128] Immunomodulators and anti-TNF agents have been associated with hematologic and dermatologic malignancies.[102]

Thiopurines and MTX appear to increase the risk of non-melanoma skin cancer both during active use of the drug and possibly after the medication is discontinued.[129-132] Patients exposed to anti-TNF agents may be at increased risk of melanoma.[131] To detect skin cancer, IBD patients on maintenance treatment with immunomodulators and/or biologic agents are yearly referred to a dermatologist for full body examination.

Patients treated with thiopurines have a three- to fivefold increased risk of developing lymphoma, which is probably the most feared complication.[129] This increased risk of lymphoma is also related to longer disease duration and older age, particularly in men.[129] Also, anti-TNF treatment and in particular combination treatment with anti-TNFs and thiopurines have also been associated with lymphoma development.[133,134] In addition, combination treatment with thiopurines and anti-TNFs has been associated with the hepatosplenic T-cell lymphoma, especially in young males.[129,135] There is no specific prevention of immunosuppressive induced hematologic malignancies and the only preventative strategy is to limit the use and duration of immunosuppressive drugs, especially in high risk patients (men older than age 65 years).[112]

Several studies suggest that IBD women are at increased risk of cervical dysplasia and cervical cancer caused by the human papillomavirus (HPV), but the role of disease characteristics and immunosuppression in the pathogenesis is unclear.[136-140] Female patients are encouraged to adhere to cervical screening programs.

CLINICAL DECISION MAKING

Many new IBD drugs targeting different immunological pathways have been, and still are being, developed to increase the chance of patients responding to a treatment. The majority of health-care costs for IBD are explained by medical therapies and thus there is a clear need for optimal use of different drugs to avert under treatment and overtreatment. There are multiple barriers to overcome, in order to achieve the ultimate goal: select the right drug for the right patient at the right time. Physicians prescribing immunosuppressive treatment to IBD patients are faced with several issues: 1) Selection of an appropriate and effective treatment strategy for the patient; 2) Avoid adverse events and (long-term) disease-related and treatment-related complications; 3) Health-care costs.

Clinical decision-making needs to be adapted to patients' age, sex and disease characteristics and correct phenotyping of the patient before initiation, followed by re-evaluation and modification of the therapeutic strategy when treatment goals are not (yet) reached. Treatment escalation or optimization should be considered in patients with inadequate response or intolerance. These optimization strategies include dose increase, shortening of treatment interval, step-up from local to systemic treatment, addition of therapy or switch to a different treatment. Disease monitoring but also TDM has allowed for early detection and prevention of loss of response, thereby optimizing drug use and cost-effectiveness. In patients that achieve sustained remission, safety concerns like adverse events, infections and cancer but also health-care costs and quality of life prompt de-escalation strategies. These

strategies comprise dose-reduction, drug switch to local treatment or step-down treatment, drug withdrawal in combination treatment or complete cessation, but also less stringent monitoring. The risk of relapse and complications should be balanced against the risk of severe adverse events and different aspects of quality of life.

Standard treatment typically depends on the extent of involvement en disease severity. However, classification based on clinical symptoms does not always predict the disease course of the patient. Despite significant efforts, also in the genetic field, there is on-going uncertainty when it comes to predicting therapy response in the individual patient. Treatment response and (short-term) adverse events have been widely studied in randomized controlled trials (RCTs), performed under strict conditions and selection of patients. Therefore, their external validity is limited as the patient population in clinical practice is more heterogeneous with different disease characteristics, age categories, comorbidities, and less stringent monitoring. Studies in real-life clinical practice are essential in visualizing actual effectiveness, drug survival and (long-term) safety aspects of treatment. In addition, data from real-life clinical cohorts may also identify more rare risk factors for treatment failure and adverse events and thereby yield new targets for treatment optimization to improve IBD treatment in the future.

AIMS AND OUTLINE OF THIS THESIS

This thesis aims to provide insight into several safety aspects and opportunities with current immunosuppressive treatment in inflammatory bowel disease. The thesis is divided in three sections.

The first part of this thesis addresses monitoring efficacy and safety of immunosuppressive treatment in inflammatory bowel disease. To increase the chance of patients responding to a treatment, it is of crucial importance to identify patients that are prone to treatment failure and could benefit from treatment modification. TDM of drug metabolites as well as laboratory monitoring of leucocytes and liver enzymes both add to the success of thiopurine therapy, since optimization of dosing to achieve optimal disease response and avoidance of toxicity are essential components. Monitoring strategies need to balance the benefits of timely adjustment of therapy and limit the burden of monitoring. **Chapter 2** describes a cohort study evaluating the clinical relevance and prognostic value of a routinely established skewed thiopurine metabolism. Continuing on this, in **Chapter 3** we describe the uncommon adverse event of myelotoxicity related to extremely high concentrations of 6-MMPR in patients receiving conventional thiopurine derivatives. In **Chapter 4** we investigate drug survival of low-dose thiopurine-allopurinol combination therapy. Furthermore, we explore risk factors for treatment cessation of this thiopurine optimization strategy. In **Chapter 5** we assess incidence rates and clinical consequences of myelotoxicity and hepatotoxicity (i.e. laboratory toxicity) within current laboratory regimen in thiopurine maintenance therapy. Special consideration

is required in female IBD patients, as the sex of the patient can have profound influences on drug metabolism and efficacy. **Chapter 6** focuses on drug survival of adalimumab and possible other sex differences in a cohort of patients with CD.

Part two of this thesis focuses on one of the extra-intestinal neoplastic complications in IBD patients: cervical dysplasia and cancer. Cervical cancer is the fourth most common type of cancer in women worldwide, caused by a persistent HPV infection. The risk of cervical dysplasia and cancer in IBD women has been studied, but results are conflicting and most of these cohorts lack details on drug exposure and longitudinal follow up. In **Chapter 7** we assess prevalence and persistence of cervical dysplasia and cancer in a large cohort of IBD women from the Dutch IBD Biobank compared to the general population. We evaluate whether certain IBD disease characteristics are associated with cervical abnormalities. It is hypothesized that chronic use of immunosuppressive drugs is a risk factor for cervical dysplasia and cancer but the association is not well understood. Therefore, we aim to assess the role of exposure to immunosuppressive drugs in development of cervical dysplasia and cancer in large cohort of IBD women in **Chapter 8**.

In the third part of this thesis we investigate rectal tacrolimus as a new treatment option for ulcerative proctitis. The majority of UC patients present with proctitis and therefore local treatment with suppositories are an attractive treatment option as systemically administered drugs are often associated with adverse events and higher costs. In **Chapter 9** we assess the efficacy of topical tacrolimus compared to topical beclomethasone as an induction therapy for ulcerative proctitis in a randomised controlled trial. Finally in **Chapter 10** the main findings of this thesis are summarized and discussed.

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PART I

RISKS IN IMMUNOSUPPRESSIVE TREATMENT

CHAPTER 2

ROUTINELY ESTABLISHED SKEWED THIOPURINE METABOLISM LEADS TO A STRIKINGLY HIGH RATE OF EARLY THERAPEUTIC FAILURE IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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ABSTRACT

Background: The conventional thiopurines azathioprine and mercaptopurine are considered maintenance immunosuppressive drugs of choice in the treatment of inflammatory bowel disease (IBD). Unfortunately, treatment is often discontinued due to adverse events or refractoriness, retrospectively associated with high levels of the thiopurine metabolites 6-methylmercaptopurine ribonucleotides (6-MMPR). Patients with a clinically "skewed" thiopurine metabolism may be particularly at risk for therapy failure. We determined the predictive value of this pharmacological phenomenon in IBD patients during regular thiopurine therapy.

Methods: Clinical effectiveness and tolerability of weight-based thiopurine therapy were determined in all IBD patients displaying a skewed metabolism (ratio 6-MMPR/6-thioguaninenucleotide (6-TGN) >20). All samples were routinely assessed between 2008-2012, as part of standard clinical follow-up after initiation of conventional thiopurine therapy.

Results: Forty-one (84%) out of 49 included IBD patients discontinued thiopurines (55% female, 53% with Crohn's disease) with a median duration of 14 weeks (range 7-155). The majority of patients with a skewed metabolism discontinued thiopurines due to adverse events (55%) or refractoriness (12%). The most commonly observed adverse event was hepatotoxicity (18 patients, 37%). Median 6-TGN level was 159 pmol/8x10^8 RBC (range 46-419), median 6-MMPR level was 11020 pmol/8x10^8 RBC (range 3610-43670) and the median 6-MMPR/6-TGN ratio was 72 (range 29-367). Thiopurine therapy failure was associated with a ratio above 50 ($p<0.03$). Hepatotoxicity occurred more frequently in patients with an extremely skewed metabolism (6-MMPR/6-TGN ratio >100) ($p<0.01$).

Conclusions: This study demonstrates that a routinely established skewed metabolism is a major risk factor for future thiopurine failure in IBD patients. These observations imply that routine thiopurine metabolite measurements may be used as a prognostic tool to identify those patients with an aberrant skewed metabolism at an early stage, possibly benefitting from therapy adjustments.

INTRODUCTION

Conventional immune-modulating thiopurine therapy, consisting of azathioprine (AZA) and mercaptopurine (MP), has proven successful in the treatment of inflammatory bowel disease (IBD) for induction treatment, but primarily for maintenance of remission. It is therefore considered to be the immunosuppressive drug of choice.[1-3] Unfortunately up to 50 percent of patients discontinue thiopurine therapy within 2-years after initiation, mainly due to the development of intractable adverse events.[1-4] Azathioprine and MP are both pharmacologically inactive pro-drugs, which are converted by three major competing enzymatic pathways to produce, amongst others, the metabolites 6-thioguanine nucleotides (6-TGN) and 6-methylmercaptopurine ribonucleotides (6-MMPR). In several studies and a meta-analysis, it has been demonstrated that 6-TGN are the pharmacologically active metabolites predominantly responsible for the immunosuppressive effects, and that these are correlating with clinical effectiveness.[5-7] Conversely, the metabolites 6-MMPR have been associated with therapy refractory disease, induction of hepatotoxicity and other adverse events.[7-9] Moreover, a 6-MMPR/6-TGN ratio above 20 is associated with therapeutic inefficacy.[10] Recent studies conclude that 15-30 percent of patients discontinue thiopurine therapy due to adverse events[8], allegedly related to a skewed thiopurine metabolism profile.[11] These so-called 'ultramethylators' preferentially produce excessive amounts of the potentially toxic 6-MMPR metabolites, whereas favorable 6-TGN levels remain below therapeutic range.[12-14] These patients are particularly at risk of thiopurine failure due to (hepato)toxicity or ineffectiveness. Although the enzymatic pathways leading to the formation of this grossly elevated 6-MMPR level have partly been unraveled, the prognostic clinical value of a skewed thiopurine metabolism for successful thiopurine therapy in IBD patients remains to be assessed.[15] The aim of this study was to establish the clinical relevance of a routinely established aberrant skewed thiopurine metabolism in IBD patients by determining the tolerability and effectiveness of ongoing treatment in these patients.

METHODS

Study design

The data of this retrospective study were based on a 4-year inception cohort from January 1st 2008 until December 31st 2011. The Pharmacy department of the VU University Medical Center (Amsterdam, The Netherlands) operates as the sole department for determination of metabolite concentrations of 6-TGN and 6-MMPR. Their database was searched to identify all patients demonstrating a skewed thiopurine metabolism, arbitrarily defined as a 6-MMPR/6-TGN ratio above 20, within the predefined study period.[12-14] These patients were cross-checked with the IBD databases and the patient registration system of our tertiary referral hospital (VU University Medical Center). This database was further scrutinized with ascertainment of (digital) chart review to identify only those IBD patients in whom conventional thiopurine therapy was initiated and displayed a skewed metabolism detected on initial *routine* determination,

generally 4 to 12 weeks after initiation of thiopurine therapy as part of our standard local protocol.[1,12] So, essentially, all consecutive IBD patients showing a skewed metabolism of thiopurines were included for analysis. Patients were excluded from analysis who were referred for thiopurine metabolite measurements, during the study period, for specific therapy related problems (adverse events, clinical refractoriness or non-compliance), and therefore lacked the initial routine thiopurine determination. As per local treatment guidelines all patients were monitored by an IBD-specialized nurse in the first 3 months of thiopurine initiation as well as during follow up. The minimal follow-up time was 1 year (till December 31st 2012). Through chart review, we retrospectively determined patient characteristics, treatment characteristics, effectiveness and tolerability of thiopurine therapy. Patients with a history of hepatitis B, C or D, history of severe pancreatitis, impaired hepatic function (>2 times the upper limit of normal of alkaline phosphatase (AP), gamma-glutamyltransferase (γ-GT), and/or alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities) at baseline and/or persistent bone marrow suppression (leukocyte count <3.5x10^9/L or platelet count <100 x 10^9/L) at baseline, were excluded from analysis. The upper limits for liver tests were defined as AP 120 U/L, γ-GT 55 U/L, ALT 45 U/L and AST 35 U/L.

Patient characteristics

Standard demographic and disease-related data were collected, including: gender, weight, age at diagnosis, duration of IBD in years at initiation of thiopurine treatment and surgical history. All patients were classified according to the Montreal classification.[16,17]

Treatment characteristics

Azathioprine and MP were prescribed according to Dutch IBD and ECCO guidelines, based on individual weight (targeted at 2-2.5 mg/kg for AZA, and 1-1.5 mg/kg for MP).[16,18] The local start-up protocol for thiopurines includes low dose prescription in the first week, followed by full dose therapy following ascertainment of tolerance, clinically and in blood in order to avoid (severe) leukopenia in patients with non-functional thiopurine S-methyltransferase (TPMT) variants. Full blood count, renal function, ALT, AST, AP and/or γ-GT performed at initiation of therapy (baseline) and at the time of metabolite assessment were documented. The following variables regarding the use of thiopurines were collected: type of thiopurine (AZA or MP), dosage and age at initiation of thiopurine therapy. Furthermore, data regarding the use of concomitant IBD related medication such as 5-ASA (5-aminosalicylic acid), steroids and biologicals were documented in order to reflect the level of complexity of enrolled IBD patients**.** In particular the use of systemic corticosteroids prescribed for an IBD indication, that exceeded the estimated thiopurine induction of remission period of 3 months. Patients were excluded from the study design when thiopurines were primarily prescribed for a non-IBD indication. Patients that initiated 5-ASA compounds simultaneously at the time of thiopurine initiation were excluded from analysis. Patients with concomitant use of allopurinol at baseline, angiotensin converting enzyme-inhibitors, trimethoprim, cimetidin or indomethacin were excluded from analysis as these drugs may interact with thiopurine metabolism.[3,19,20]

Thiopurine metabolite measurements

A skewed metabolism was defined as a 6-MMPR/6-TGN ratio above 20, which closely reflects the ratio of target concentrations for both metabolites, i.e. 6-MMPR <5700 pmol/8x10^8 red blood cells (RBC) and 6-TGN >235 pmol/8x10^8 RBC.[5,14] The extent of ultramethylation was arbitrarily defined as mild (ratio 20-50), moderate (ratio 50-100) and severe (ratio >100). The measurements were performed in the laboratory for Clinical Pharmacology and Pharmacy at the VU University Medical Center. The levels of 6-TGN and 6-MMPR metabolites in RBCs were measured by a validated high performance liquid chromatography assay, according to the method described by Dervieux and Boulieu.[21,22,23,24] This method has been used in the laboratory for more than 8 years to monitor thiopurine metabolites in more than 10.000 samples from Dutch hospitals. The accuracy is 107%, the between-assay imprecision is 6,7%. The laboratory participates in the thiopurine proficiency testing program of the Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology in the Netherlands. Blood samples for 6-TGN and 6-MMPR metabolite measurements were obtained at least 4 weeks after initiation of AZA or MP in order to determine steady-state levels.[25,26] As part of the local treatment protocol, metabolites were generally measured 8 weeks after therapy onset as a routine.

Effectiveness and tolerability

Clinical effectiveness was assessed by means of a global physician's assessment, indicative of quiescent disease, supported by systemic steroid withdrawal for >3 months and the absence of inflammation on laboratory findings, such as C-reactive protein (CRP) and/or faecal calprotectin, and radiological, endoscopic and histopathological reports (if present). Refractoriness was defined as the inability to achieve the above mentioned criteria for clinical effectiveness and was subdivided into: primary non-response after 3 to 6 months of treatment or loss of therapeutic response after more than 6 months of treatment. Sustained clinical benefit was defined as ongoing use of the initiated thiopurine therapy at the time of data collection at the last outpatient contact before the end of study period, or intentional discontinuation of successful thiopurine therapy at patient's request at any date before end of study. Failure was defined as cessation of thiopurine therapy due to refractoriness, or in consequence of the occurrence of any intolerable AE. Initiation of allopurinol and/or biologicals therapy concomitant with a thiopurine was also considered therapy failure. An adverse event to thiopurine administration was defined as any reaction, side effect or untoward event that occurred during the course of thiopurine treatment. Adverse events were subdivided, according to Dutch IBD guidelines, into gastro-intestinal complaints like nausea and vomiting, general malaise, arthralgia, dermatological eruptions, hepatotoxicity, myelosuppression, pancreatitis, lymphomas, infectious complications and others such as alopecia or myalgia.[16] Hepatotoxicity was defined as twice or more the upper limit of normal of ALT, AST, AP and/or γ-GT activities, supported by histopathological findings, if present. Furthermore, with retrospective ascertainment of chart review, patients were excluded in case the diagnostic work-up of the treating physician demonstrated signs of viral, auto-immune or steatotic causes of hepatotoxicity. Myelosuppression was defined as a leukocyte count <3.5

x 10^9/L and/or platelet count <100 x 10^9/L, according to the Common Terminology Criteria for Adverse Events (version 3.0).

Data analysis

Statistical analysis was performed using SPSS for Windows version 20.0. Quantitative data were expressed as means with standard deviations for continuous data, percentages for categorical data, or in case of non-normal distributions as medians with range. Group differences for categorical variables were assessed using Chi-square or Fisherman's test. Group differences for continuous variables were assessed using the ANOVA, Mann-Witney or Students T-test for paired or individual data. Variables possibly predicting outcomes of thiopurine therapy discontinuation were investigated by multivariate regression analysis. For proper comparison with existing literature, which often utilizes the Lennard method to determine the metabolite levels, 6-TGN levels were recalculated by dividing the 6-TGN outcome by a converting factor of 2.6.[24,27] The 6-MMPR metabolite outcomes are comparable in both methods. Multiple documented AEs in one patient were separately counted, due to which the summed percentages of singular AE-groups may exceed 100 percent. P values <0.05 were considered statistically significant.

RESULTS

Patient characteristics

Of the estimated total IBD population of approximately 2000 patients treated at the VU University Medical Center, around 800 patients (i.e. 40%) were treated with thiopurine immunosuppression and 140 patients treated with conventional thiopurines displayed an aberrant thiopurine metabolism (6-MMPR/6-TGN ratio above 20) between January 1st 2008 and December 31st 2011. Seventy-four patients (53%) were excluded due to alternative indicative factors for thiopurine metabolite assessment (i.e. suspected thiopurine induced AE or clinical refractoriness) or insufficient data to deduce the indication for metabolite assessment. Twelve patients were excluded from analysis based on uncertainty of definitive IBD diagnosis or concomitant use of ACE-inhibitors (n=2). One patient was formerly diagnosed with an autoimmune hepatitis with severe liver test abnormalities and was therefore excluded from analysis. Four patients were lost to follow up, leaving a total of 49 patients in which metabolite levels were determined as a routine for further analysis. Twenty-seven patients were female (55%) and the median age of diagnosis was 27 years (range 18-79). Twenty-six patients (53%) were diagnosed with Crohn's disease (CD), mainly located in the terminal ileum and colon region. Twenty-three patients (47%) were diagnosed with ulcerative colitis (UC). The majority of UC patients (52%) had an extensive colitis. Baseline patient demographics and disease characteristics are depicted in **Table 1.**

Table 1. Patient and Treatment Characteristics

	N = 49
Gender M : F (n) (%)	22 : 27 (45 : 55)
CD Patients (n) (%)	26 (53)
UC Patients (n) (%)	23 (47)
Median age at diagnosis (years) [range]	27 [18-79]
Smoking status (active/former : never)	21 : 28
CD – Montreal classification: Age at diagnosis <16 years 17-40 years >40 years	3 20 3
CD - Montreal classification: Behavior non-stricturing, non-penetrating stricturing penetrating	10 2 14
CD - Montreal classification: Localization ileal colonic ileocolonic upper GI disease	6 6 11 3
UC - Montreal classification: Extent ulcerative proctitis left-sided ulcerative colitis pancolitis	1 10 12
History of IBD related surgery (n) (%)	14 (29)
AZA : 6-MP (%)	4 : 45 (8 : 92)
Median thiopurine dose (mg/kg) AZA (n = 3, 1 missing) [range] 6MP (n = 45) [range]	1.98 [1.69-3.13] 1.20 [0.60-2.08]
Systemic steroids >3 months at induction of remission (n) (%) Median duration (weeks) [range]	10 (20) 41 [15-125]
5-ASA during thiopurines (n) (%)	26 (53)
Biological at induction (n) (%)	6 (12)

F, Female; M, Male ; CD, Crohn's Disease; UC, Ulcerative colitis; 5-ASA, aminosalicylates.

Treatment characteristics

At the time of thiopurine metabolite measurement, 45 patients (92%) were treated with MP with a median dose of 1.20 mg/kg (range 0.6-2.08) and 4 patients (8%) with AZA with a median dose of 1.98 mg/kg (range 1.69-3.13). Prolonged use of systemic glucocorticoids prescribed for an IBD indication, at remission-induction that exceeded the estimated period of 3 months, occurred in 9 patients with a total median usage of 41 weeks (range 15-125). Twenty-six patients (53%) were using 5-ASA compounds as maintenance strategy alongside thiopurine therapy, generally in case of UC (20 patients, 77%). Twelve (24%) patients had documented use of AZA in the past. Ten of these patients switched to MP and two patients were re-challenged with AZA. Reasons for historical discontinuation of preceding AZA-therapy were ineffectiveness (n=6), intolerance (n=2), or issues of compliance (n=1). Two patients discontinued AZA at

their own initiative and two patients due to prolonged remission of disease. All therapeutic characteristics are also depicted in **Table 1.**

Routinely established thiopurine metabolite measurements

All metabolite concentrations are depicted in **Table 2**. Overall, routine metabolite level determination was performed after a median duration of 8 weeks (range 4-22) after initiation of thiopurine treatment. Nine patients with a skewed metabolism of MP showed metabolite levels during the previous weight-based AZA usage within normal ranges.

Table 2. Thiopurine Metabolites and Laboratory Results

6-TGN pmol/8x10^8 RBC (n=49)	159 [46 - 419]
6-MMPR pmol/8x10^8 RBC (n=49)	11020 [3610 - 43670]
Ratio 6-MMPR/6-TGN (n=49)	72 [29 - 367]
CRP mg/l (n=48)	7.8 [<2.5 – 63.7]
Hb g/dl (n=49)	7.8 [5.8 – 9.8]
Erythrocyte count 10^3/µl (n=47)	4.1 [3.1 – 5.3]
Platelet count 10^3/µl (n=47)	332 [102 – 603]
Leukocyte count 10^3/µl (n=48)	6.4 [1.9 - 22.2]
MCV (n=46)	91 [79 - 105]
Creatinine (n=48)	67 [37-100]
AP U/l (n=46)	70 [41 - 134]
γ-GT U/l (n=45)	26 [7 - 128]
AST U/l (n=35)	26 [14 - 98]
ALT U/l (n=48)	37 [11 – 339]

Laboratory results at the moment of thiopurine metabolite determination. 6-thioguaninenucleotide (6-TGN), 6-methylmercaptopurine ribonucleotides (6-MMPR), C-reactive protein (CRP), Haemoglobin (Hb), Mean corpuscular volume (MCV), Alkaline phosphatase (AP), gamma-glutamyltransferase (γ-GT), aspartate aminotransferase (AST), Alanine aminotransferase (ALT). Results are reported as median with ranges.

Therapeutic effectiveness and tolerability of thiopurines

From the total cohort of skewed thiopurine metabolizers, 84% of patients (41/49) discontinued thiopurine monotherapy. The median duration of thiopurine use in the patients who failed thiopurine therapy was 14 weeks (range 7-155). Nineteen patients (39%) discontinued treatment within 12 weeks after thiopurine initiation (**Figure 1a**).

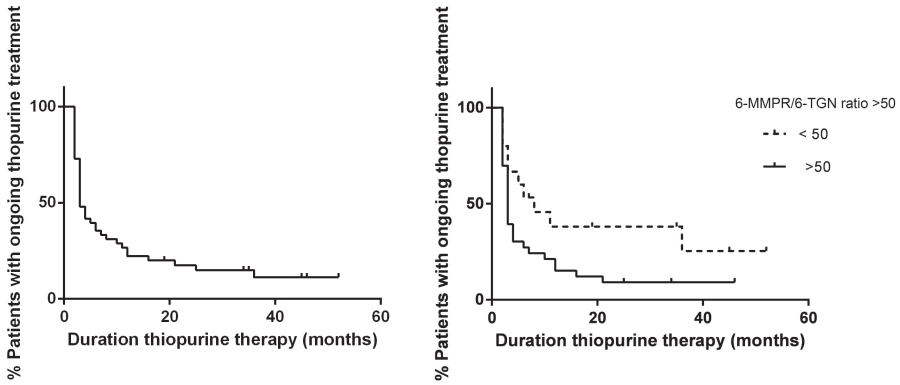

Figure 1a-b. Kaplan-Meier curves regarding ongoing thiopurine use in all IBD patients in months. The Y-axis represents the percentage of patients continuing thiopurine therapy over time until the end of follow up. Figure 1b is corrected for the 6-MMPR/6-TGN ratio.

All reasons for therapy discontinuation are depicted in **Table 3**. The main reason for thiopurine therapy failure was intolerance (n=27, 55%).

Table 3. Therapeutic effectiveness and tolerability of thiopurines

	N = 49 , n (%)
Patients discontinuing thiopurine therapy	41 (84)
Reason thiopurine therapy failure	27 (55)
Intolerance	6 (12)
Refractoriness	4 (8)
primary non-response	2 (4)
loss of response	4 (8)
Early treatment intensification (due to severe clinical course)	3 (6)
Intentional discontinuation	1 (2)
Insufficient data	
Alternative therapy	18 (37)
Allopurinol combination therapy	9 (18)
TG	3 (6)
MTX	4 (8)
Addition biological drug	4 (8)
None	2 (4)
Study enrollment	1 (2)
Surgical treatment	

TG (thioguanine), MTX (methotrexate)

Common AEs were hepatotoxicity (n=18), gastrointestinal complaints (n=11) and general malaise (n=11) including fatigue, headache and dizziness. Eight patients discontinued therapy due to laboratory signs of myelosuppression, of which five patients displayed an isolated leukopenia and three patients showed a pancytopenia. Other AE comprised dermatological abnormalities (n=2). (**Figure 2a-2c**). No pancreatitis, lymphomas, infectious complications or mortality were reported. In several patients more than one AE occurred.

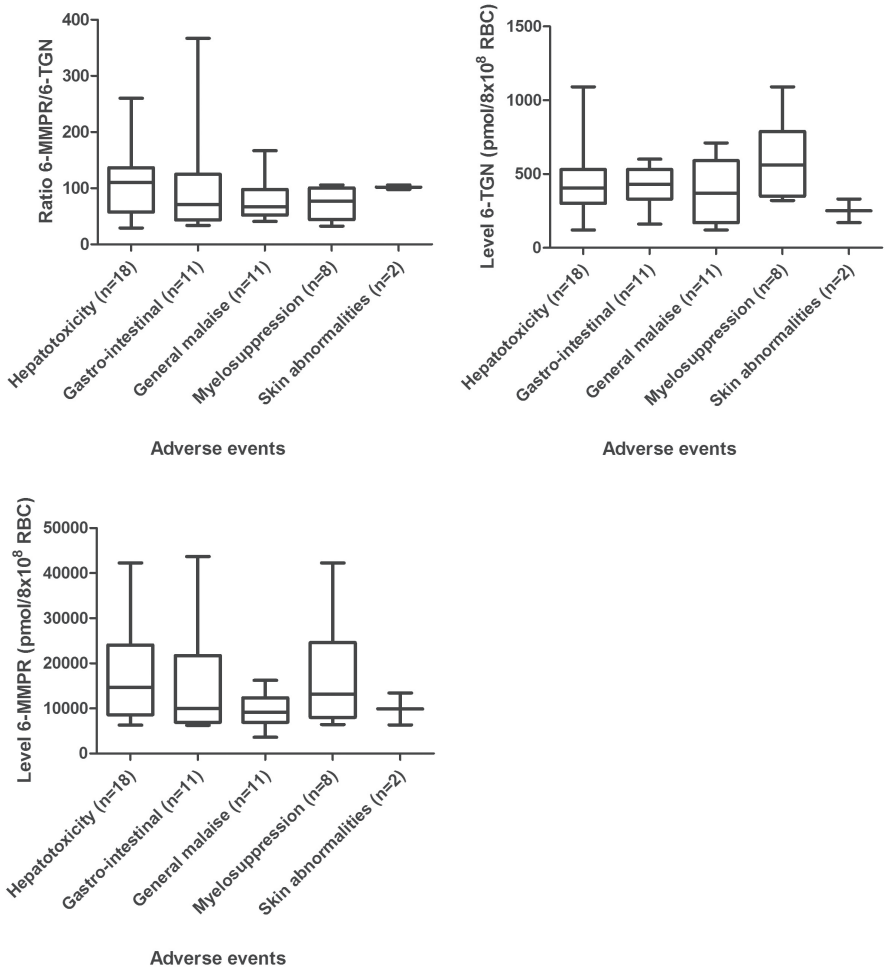

Figure 2a-c. Box-whisker plots of discontinuation due to adverse events in IBD patients with a skewed metabolism on conventional thiopurines with corresponding 6-MMPR/6-TGN ratios (2a), 6-TGN (2b) and 6-MMPR (2c) metabolite levels. Statistical analysis in these small groups did not show statistical significant differences between adverse events and metabolite levels.

Altogether we observed 50 AE in 36/49 patients (74%), leading to therapy discontinuation in 27 patients. Overall adverse events occurred more frequently in patients discontinuing thiopurine therapy ($p < 0.02$). In this study, all patients (n=9) with prolonged or maintenance use of (low-dose) steroids discontinued thiopurine treatment over time. Patients with successful thiopurine therapy (n=9) achieved remission without prolonged use of steroids. Patients discontinuing treatment due to thiopurine refractoriness, failed therapy with a median duration of thiopurine use of 22 weeks (range 12-155) compared to a median duration of 11 weeks (range 7-108) in the AE group ($p<0.05$). The consequences of failure to thiopurine treatment are depicted in **Table 3**. In sixteen patients failure led to withdrawal of

thiopurines. Of these sixteen patients, 12 patients received escape treatment being tioguanine (6-TG) and methotrexate and four patients with UC achieved or maintained remission on monotherapy 5-ASA without alternative therapy adjustments. One patient underwent a total proctocolectomy with permanent ileostomy. No mortality was reported.

The addition of allopurinol in eighteen patients, generally 100mg once daily, alongside a 3 to 4 times reduced thiopurine dose led to restoration of the aimed 'optimal' ratio <11 of metabolites in all patients.[28] Initial median 6-MMPR/6-TGN ratio was 90 (range 41-367) and median 6-MMPR/6-TGN ratio was 1.8 (range 0.1-7.3, 1 missing) after the initiation of allopurinol. Although myelosuppression remained in one patient, no other AE were reported, especially no hepatotoxicity. Concomitant therapy of thiopurines with allopurinol induced clinical remission in 13 out of 18 (72%) patients. Five patients were unable to achieve clinical remission with thiopurine and allopurinol combination therapy, leading to the initiation of 6-TG in one patient and anti-TNF therapy in four patients.

Metabolite monitoring and therapy failure

The absolute (height of) concentration of either 6-TGN or 6-MMPR metabolites did not show statistically significant differences between patients continuing and stopping treatment. Median 6-MMPR/6-TGN ratio was 35 (range 33-235) in the patients continuing thiopurines despite a skewed metabolism, versus 82 (range 29-367) in patients discontinuing treatment ($p<0.09$). Fifteen patients (31%) displayed a relatively mild skewed thiopurine metabolism (ratio 20-50) of which ten (67%) patients discontinued treatment. Fifteen patients (31%) demonstrated a relative moderate ultramethylation (ratio 50-100) and fourteen (93%) of these patients discontinued treatment. Nineteen patients (38%) had a strongly skewed metabolism (ratio >100), 17 (89%) of these patients failed treatment (**Table 4).** Patients with a ratio >50 discontinued thiopurine treatment more often than patients displaying relatively mild ultramethylation ($p<0.03$) (**Figure 1b**).

Table 4. Pharmacology of thiopurine use and (dis)continuation of therapy

	Continuation n=8	Discontinuation n=41	P-value
Median level 6-TGN (pmol/10e8RBC) [range]	167 [96-294]	158 [46-210]	0.55
Median level 6-MMPR (pmol/10e8RBC) [range]	11270 [4010-23460]	11020 [3610-43670]	0.48
Median 6-MMPR/6-TGN Ratio [range]	35 [33-354]	82 [29-419]	0.09
Median duration thiopurine therapy (weeks) [range]	169 [83-225]	14 [7-155]	<0.0001
Level of ultramethylation			
Mild: 20-50 (n) (%)	5 (63)	10 (24)	<0.0001 (0.03*)
Moderate: 50-100 (n) (%)	1 (12)	14 (34)	<0.0001 (0.22*)
Severe: >100 (n) (%)	2 (25)	17 (42)	<0.0001 (0.38*)

Routine measurements of thiopurine metabolites. 6-thioguaninenucleotide (6-TGN), 6-methylmercaptopurine ribonucleotides (6-MMPR), Level of ultramethylation (6-MMPR/6-TGN, according to Lennard's method). Results are reported as median with ranges. *Level of ultramethylation compared to the remaining groups.

Patients developing hepatotoxicity were more often extreme skewers (p<0.01) than patients in whom no hepatotoxicity was present. No variables were identified that could predict failure of monotherapy with thiopurine or specific adverse events such as hepatotoxicity or myelosuppression, including Montreal classification, specific thiopurine derivate, dosage, previous treatment characteristics or the absolute level of 6-TGN and 6-MMPR metabolites.

Sustained clinical benefit

Thiopurine therapy was continued in 8/49 patients (16%). Four patients achieved clinical remission, three patients improved clinically and one patient intentionally discontinued treatment at own discretion. The median duration of thiopurine therapy in patients with ongoing treatment at the end of follow up was 169 weeks (range 83-225) (**Figure 1a**).

DISCUSSION

In this retrospective, single-centre cohort study of almost fifty thiopurine ultramethylating IBD patients, it was demonstrated that a skewed metabolism, routinely established per local treatment protocol, is critical to predict intolerability and ineffectiveness of conventional thiopurine treatment. Eighty-four percent of these patients discontinued thiopurine therapy most often due to the development of intractable AEs, especially hepatotoxicity. These findings underline that a skewed metabolism (6-MMPR/6-TGN ratio above 20) is a prognostic risk factor for thiopurine therapy failure.

In several trials and a meta-analysis it has been demonstrated that conventional thiopurine therapy is effective in the treatment of IBD patients for induction treatment, but primarily for maintenance of remission.[1-3] In recent studies, it has been shown that differences in response to and tolerance of thiopurines are most likely to result from individual variations in drug metabolism in which high 6-MMPR and 6-TGN concentrations are correlated with the occurrence of (hepato)toxicity and myelosuppression, respectively.[5,7-9,25] Furthermore a 6-MMPR/6-TGN ratio above 20 is associated with therapeutic inefficacy.[5,6,10] Several studies demonstrated that up to 46% to 83% of IBD patients discontinued conventional thiopurine treatment, usually in the first 2-3 years.[4,8,28,29] The results of our study, consisting exclusively of ultramethylating IBD patients demonstrate that a substantially higher percentage of these patients (84%) discontinued thiopurine treatment at an earlier stage. Conventional thiopurine therapy was effective in only 16% of patients with a skewed metabolism, revealed by routine measurements. Although this small group of patients benefitted from conventional thiopurine monotherapy up to several years, this appeared the exception more than the rule. In previous studies in average IBD populations, over 28% of patients discontinued thiopurine therapy due to AE, allegedly related to a skewed thiopurine metabolism profile.[1,4,8,30] In this study the rate at which patients discontinued therapy due to AE was nearly twice as high (55%). The majority of patients discontinued therapy due to AE, mainly thiopurine induced hepatotoxicity (37% of all patients), and this was associated with a strongly skewed metabolism. These results are in line with the hypothesis that induction of hepatotoxicity and other AEs are specifically associated with high 6-MMPR levels.[7-9] Moreover, eight patients discontinued treatment due to thiopurine-induced myelotoxicity. This finding suggests that the risk of developing thiopurine induced haematological events is not exclusively related to 6-TGN metabolites, but that also 6-MMPR metabolites play an important role in this phenomenon: 6-MMPR metabolites can inhibit the *de novo* purine synthesis leading to a depletion of purines which are essential elements for DNA and RNA formation and may interfere with cellular replication.[5,25,31]

In the past, it has been suggested that high TPMT activity leads to high 6-MMPR concentrations and was therefore associated with therapy failure. However, van Egmond et al[14] found that differences in TPMT enzyme activity did not predict preferential 6-MMPR metabolite production and other factors had to contribute to this aberrant and unfavourable metabolism. The phenomenon of ultramethylation is generally discovered by the occurrence of insurmountable AEs or lack of clinical response, sometimes after several months of treatment, leading to metabolite measurements helping to unravel its cause. Therefore it is not surprising that ultramethylators discontinue thiopurine treatment more frequently than patients without preferential 6-MMPR metabolite generation. In all our 49 patients, metabolites were measured as a routine after initiation of thiopurine therapy, thus making an overestimation of patients less plausible. This study confirmed that a skewed metabolism is a strong risk factor for future thiopurine treatment failure. Determination of thiopurine metabolite concentrations as a routine to identify patients with a skewed metabolism may result in the reduction of potentially redundant and unnecessary AEs by early therapy adjustments. This combination of findings provides some support for the conceptual premise that adjustment of treatment in the

form of thiopurine dose-reduction in combination with allopurinol is an effective alternative immunosuppressive strategy.[8,13]

There are some limitations to our study. Firstly, numerous patients received previous (thiopurine) treatment before being referred to our hospital, resulting in different therapy strategies in the past. Furthermore, several patients received thiopurine treatment with a concomitant biological drug, conceivably representing a more complex type of IBD in this group, possibly conflicting with clinical outcomes in the present study. Secondly, we used the first assessment of thiopurine metabolites for each patient. Although all metabolites were routinely determined, the time of measurement after thiopurine initiation was to some extent variable. Nine patients with a skewed metabolism of MP showed normal metabolite levels during previous AZA usage. Whether the explanation for this finding can be assigned to dosage, a different thiopurine metabolism, for example being caused by a different first-pass effect, or increasing metabolite levels over time due to co-medication or disease activity remains to be elucidated.[20]

In conclusion, we observed that IBD patients with a skewed metabolism of conventional thiopurines, established during a routine measurement, with corresponding high 6-MMPR/6-TGN ratios discontinued therapy early and at an excessively high rate of 84%, mostly due to AEs, with 6-MMPR-associated hepatotoxicity being the commonest culprit. For a more accurate risk stratification, thiopurine metabolite levels may be routinely measured in patients starting conventional thiopurines, identifying those benefitting from therapy adjustments early.

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CHAPTER 3

THIOPURINE INDUCED LEUKOPENIA CAUSED BY ELEVATED 6-MMPR LEVELS: CLINICAL CHARACTERISTICS AND OUTCOME OF THERAPY OPTIMIZATION

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ABSTRACT

Background: Thiopurines have a favorable benefit–risk ratio in the treatment of inflammatory bowel disease. A feared adverse event of thiopurine therapy is myelotoxicity, mostly occurring due to toxic concentrations of the pharmacologically active metabolites 6-thioguaninenucleotides. In oncology, myelosuppression has also been associated with elevated 6-methylmercaptopurine ribonucleotides (6-MMPR). In this case series, we provide a detailed overview of 6-MMPR-induced myelotoxicity in inflammatory bowel disease patients.

Methods: We retrospectively scrutinized pharmacological laboratory databases of five participating centers over a 5-year period. Patients with leukopenia at time of elevated 6-MMPR levels (>5,700 pmol/8 x 108 red blood cells (RBCs)) were included for detailed chart review.

Results: In this case series, we describe demographic, clinical and pharmacological aspects of 24 cases of 6-MMPR induced myelotoxicity on weight-based thiopurine therapy with a median 6-MMPR level of 14,500 pmol/8 x 108 RBCs (range 6,600-48,000). All patients developed leukopenia (white blood cell count 2.7±0.9 x 109/L) after a median period of 11 weeks after initiation of thiopurine therapy (interquartile range 6-46 weeks). Eighteen patients (75%) developed concurrent anemia (median haemoglobin concentration 6.9 x 109/L) and four patients developed concurrent thrombocytopenia (median platelet count 104 x 109/L). Leukopenia resolved in 20 patients (83%) within four weeks upon altered thiopurine treatment regimen, and white blood cell count was increasing, but not yet normalized, in the remaining four patients.

Conclusion: We observed that thiopurine-induced myelotoxicity also occurs because of (extremely) high 6-MMPR concentrations in patients with a skewed thiopurine metabolism. Continued treatment with adapted thiopurine therapy was successful in almost all patients.

INTRODUCTION

Thiopurines (i.e. azathioprine and mercaptopurine) are immunosuppressive drugs that play an indispensable therapeutic role in maintaining remission in the majority of patients with inflammatory bowel disease (IBD) (primarily Crohn's disease and ulcerative colitis).[1-3] The complex metabolism of thiopurines has been largely unravelled over the years, leading to the observation that thiopurine S-methyl transferase (TPMT) plays a pivotal role in the bioavailability of the pharmacologically active end-metabolites: 6-thioguanine nucleotides (6-TGN, therapeutic window 230–450 pmol/8 × 108 red blood cells [RBC]) and 6-methylmercaptopurine ribonucleotides (6-MMPR, normal value <5700 pmol/8 × 108 RBC).[4,5] Hepatotoxicity induced by thiopurines is largely associated with the 6-MMPR metabolites, whereas myelotoxicity is mostly ascribed to high concentrations of 6-TGN, leading to apoptosis and direct cytotoxicity due to DNA strand breakage.[6-8] However, when 6-MMPR concentrations are (extremely) high, as seen in high-dose thiopurine therapy in oncological patients, or in IBD patients with a skewed thiopurine metabolism (e.g. but not solely, caused by high TPMT activity), 6-MMPR can inhibit the de novo purine synthesis, thus causing subsequent myelotoxicity.[4,9,10] Monitoring of thiopurine metabolites (6-TGN and 6-MMPR) in RBC and/or TPMT mutation analysis is becoming integrated in general IBD practice to optimize efficacy and to minimize thiopurine toxicity. Furthermore, when starting thiopurine therapy, it is advised to monitor laboratory parameters on a regular basis for early detection of toxicity.[2,3] Myelotoxicity caused by high 6-MMPR levels is believed to be an uncommon adverse event in IBD patients.[11-13] Here, we present 24 cases with 6-MMPR induced leukopenia and describe subsequently applied strategies to optimize thiopurine metabolism by preventing excessive 6-MMPR generation.

METHODS

Study design

The pharmacological laboratory databases of three tertiary referral centers in the Netherlands (VU University Medical Center, Amsterdam; Erasmus Medical Center, Rotterdam; and Maastricht University Medical Center,Maastricht) and two large teaching hospitals (Zuyderland Medical Center, Heerlen-Sittard-Geleen and Máxima Medical Center, Veldhoven) were scrutinized by an automated search over a time period of 5 years (January 1,2011–December 31, 2015) for this retrospective study. Furthermore, these databases were crosschecked with IBD databases on site.

Patient selection

The pharmacological reports of all patients using thiopurines and having at least one metabolite measurement in the selected time period were analyzed. We included all IBD patients with 6-MMPR concentrations above 5700 pmol/8 x 108 RBC for detailed chart review. Leukopenia was defined as white blood cell count below 4.0 x 109/L. Exclusion criteria were

the absence of leukopenia (i.e. white blood cell count ≥ 4.0 x 109/L), 6-TGN concentrations above normal limits (i.e. > 450 pmol/8 x 108RBC), the absence of a skewed metabolism (i.e. 6-MMPR/6-TGN ratio < 20) or the lack of laboratory measurements within 3 days prior to or after metabolite measurement.

Demographic characteristics

At time of leukopenia, we collected the following data on patient characteristics: sex, age, weight, IBD type, Montreal classification[14], specific thiopurine derivative, dosage and duration of thiopurine therapy, and concomitant medication, in particular drugs known to induce myelosuppression by itself (e.g. allopurinol[15], ACE inhibitors[8], ribavirin[16] and mesalazine[17,18], or interfere with thiopurine metabolism. Treatment strategies following (allegedly) 6-MMPR-induced leukopenia (i.e. discontinuation, dose reduction, low dose thiopurine combined with allopurinol (LDTA), or switch to thioguanine) were evaluated. When patients were admitted to the hospital with fever at time of diagnosed leukopenia, routine blood cultures and virologic tests were assessed to rule out other causes (e.g. viral infection or sepsis).

Laboratory tests

We collected the following hematologic parameters from all included patients at time of diagnosed leukopenia and 2–6 weeks after application of thiopurine optimization strategy: white blood cell count (WBC; normal range 4.0–10.0 x 109/L), hemoglobin concentration (Hb; normal range male 8.5–11.0 x 109/L, female 7.5–10.0 x 109/L), mean corpuscular volume (normal range 80–100 fL), platelet count (normal range 150–400 x 109/L), aspartate aminotransferase (reference value male ≤ 35 U/L, female ≤ 40 U/L), and alanine aminotransferase (reference value ≤ 55 U/L). Differentials of WBC were collected when determined within 3 days after diagnosed leukopenia. Furthermore, we collected thiopurine metabolites ([6-MMPR] and [6-TGN]) at time of myelotoxicity (within 3 days prior to or after diagnosed leukopenia) and during follow-up (i.e. 2–6 weeks after optimizing therapy), when available. Concentrations of metabolites were measured using a previously described method by Dervieux et al.[19] or Lennard et al.[20] The Lennard method has found the greatest application in clinical studies yet and has served as the basis for the establishment of treatment-related therapeutic ranges for thiopurine therapy.[21] Laboratory measurements of the Maastricht University Medical Center and the Máxima Medical Center were performed in the Zuyderland Medical Center. In the Zuyderland Medical Center, concentrations of metabolites were measured using the method described by Lennard until April 2013. From April 2013, the method by Dervieux was applied. Concentrations of metabolites in the other two centers were measured using the method described by Dervieux. In these centers, concentrations of 6-TGN were divided by a factor 2.6 to make them comparable to those determined by the Lennard method.[22,23] Concentrations of 6-MMPR are similar in both assays.[21]

Data analysis

All data are given descriptively or tabulated. Data are expressed as median with interquartile range (IQR), range, or as mean with standard deviation according to distribution. Metabolite

concentrations at baseline and after applying treatment optimization strategies were compared using the Wilcoxon signed-rank test. Correlations between nonparametric values were measured using the Spearman's rank order correlation test.

Ethical approval

This study was approved by the Medical Ethics Review Committee of the VU University Medical Center with file-number 2016-824.

RESULTS

Patient characteristics

A total of 24 patients (50% male, 50% female) were included with a mean age at initiation of thiopurine therapy of 44 ± 18 years. Crohn's disease and ulcerative colitis were diagnosed in nine (38%) and 15 (62%) patients, respectively. Median duration of thiopurine therapy until development of myelotoxicity was 11 weeks (IQR 6–46). All patient characteristics are summarized in **Table 1**.

Development of leukopenia

After a median period of 11 weeks after initiation of thiopurine therapy, leukopenia developed with a mean WBC of 2.7±0.9 x 109/L. In 18 patients (75%), hemoglobin decreased under the lower reference limit to a median of 6.9 x 109/L (range 3.2 – 8.4) simultaneously. Concurrent thrombocytopenia occurred in four patients (17%) with a median of 104 x 109/L (range 79 - 132). Three patients developed pancytopenia. Median 6-MMPR was 14,500 pmol/8 x 108 RBC (range 6,600-48,000) with with therapeutic 6-TGN in nine patients (38%; mean concentration 196±98 pmol/8 x 108 RBC) In the other 15 patients, 6-TGN concentrations were lower than the therapeutic cut-off level (i.e. <235 pmol/8 x 108 RBC). The median 6-MMPR/6- TGN ratio was 102 (range 24–327). The 6-MMPR/6-TGN ratio was not correlated to WBC (p=0.23), but there seemed to be a trend towards lower WBC in patients with higher 6-MMPR concentrations (r=-0.30, p=0.08). An overview of these results is depicted in **Tables 2 and 3**. Four patients (nos 6, 15, 20, and 21) were admitted to the hospital because of complicated myelotoxicity combined with fever, deep anemia, and/or worsening of IBD. Of these patients, three patients (nos 6, 15, and 21) were febrile and treated with intravenous antibiotics per local protocol. One patient (no. 20) was admitted for blood transfusion (Hb 3.2x109/L) and received three units of erythrocytes concentrate, after which the anemia resolved. Patient nos 15 and 20 were also admitted because of worsening of IBD course and received an induction course of prednisolone. In these patients, thiopurine treatment was immediately ceased.

Table 1. Demographics of included patients

No.	Sex	Age (years)	D	Montreal	Drug	Dose (mg/day)	Weight (kg)	Dose (mg/kg)	Relevant co-meds	Week of leukopeniac
1	F	55	CD	A2L2B1	MP	50	64	0.8	none	1000
2	F	80	CD	A3L1B2	MP	75	67	1.1	none	220d
3	M	33	CD	A2L3B1p	MP	75	60	1.3	none	9
4	F	24	CD	A2L1B2	MP	75	56	1.3	none	6
5	F	34	CD	A2L3B1	MP	100	87	1.1	none	110
6	M	18	CD	A2L1B1	MP	100	68	1.5	none	6
7	F	43	CD	A2L3B1	MP	100	65	1.5	mesalazine	20
8	M	21	CD	A1L3B1	MP	100	56	1.8	none	6
9	F	62	CD	A3L2B1	AZA	150	72	2.1	lisinopril	4
10	F	26	CD	A2L1B1	AZA	175	76	2.3	none	46
11	M	74	UC	E3	MP	75	71	1.1	mesalazine	12
12	M	23	UC	E3	MP	75	65	1.2	mesalazine	12
13	F	49	UC	E3	MP	75	65	1.2	mesalazine	6
14	M	50	UC	E3	MP	100	78	1.3	none	104
15	F	67	UC	E2	MP	100	75	1.3	mesalazine	6
16	F	75	UC	E2	MP	100	75	1.3	none	45
17	F	34	UC	E3	MP	100	57	1.8	none	5
18	M	31	UC	E2	MP	125	89	1.4	mesalazine	6
19	F	32	UC	E3	MP	150	92	1.6	none	12
20	M	51	UC	E3	MP	150	86	1.7	mesalazine	4
21	M	35	UC	E3	MP	150	84	1.8	none	5
22	M	63	UC	E1	AZA	125	67	1.9	none	156
23	M	51	UC	E2	AZA	150	60	2.5	mesalazine	45
24	M	34	UC	E3	AZA	200	87	2.3	mesalazine	9

M: male, F: female, D: diagnosis, CD: Crohn's disease, UC: ulcerative colitis, MP: mercaptopurine, AZA: azathioprine. a Montreal classification: A: age at diagnosis – 1: below 16 years, 2: 17-40 years, 3: above 40 years. L: location – 1: ileal, 2: colonic, 3: ileocolonic. B: behaviour – 1: non-stricturing non-penetrating, 2: stricturing, 3: penetrating, p: perianal involvement. E: extent – 1: proctitis, 2: left-sided colitis, 3: extensive colitis. b Relevant co-medications were defined as mesalazine, sulfasalazine, ace-inhibitors, trimethoprim, indometacine and ribavirine. c week of diagnosed leukopenia. d Leukopenia developed 4 weeks after dose increase.

Table 2. Laboratory parameters of patients developing myelotoxicity on thiopurine therapy due to high 6-methylmercaptopurine concentrations

CASE	At time of developing myelotoxicity								
	WBC (x109/L)	Hb (x109/L)	MCV (fL)	PC (x109/L)	AST (U/L)	ALT (U/L)	6-MMPR	6-TGN	6-MMPR/6-TGN ratio
1	2.2	7.5	122	194	130	88	19 000	139	137
2	3.7	7.1	97	294	27	38	12 500	58	216
3	3.2	8.4	101	152	-	30	13 000	296	44
4	2.7	7.7	-	225	-	100	12 000	212	57
5	3.6	4.9	-	167	-	30	36 500	139	263
6	1.7	6.8	89	234	20	36	33 000	173	173
7	2.3	6.3	112	203	-	-	48 000	279	172
8	2.0	7.8	-	269	-	14	11 000	423	26
9	2.5	7.6	122	132	69	55	22 000	215	102
10	3.7	8.5	94	311	18	15	6 600	85	78
11	3.5	6.6	110	79	51	50	7 500	308	24
12	2.6	6.6	90	329	28	52	13 000	262	50
13	3.5	7.5	-	253	-	35	13 000	169	77
14	3.9	9.6	89	249	-	181	16 000	80	200
15	1.8	7.2	98	125	29	39	46 000	273	168
16	3.8	7.3	108	267	60	68	22 000	273	81
17	3.4	7.0	106	209	-	10	36 000	110	327
18	1.8	6.6	91	409	-	69	30 000	319	94
19	2.3	6.5	99	199	-	61	37 000	262	141
20	1.6	3.2	100	417	11	9	19 000	73	260
21	0.8	5.8	-	155	111	274	13 000	65	200
22	2.2	7.4	104	82	27	38	13 000	127	102
23	3.5	8.4	90	315	47	41	10 000	223	45
24	2.5	8.1	90	204	17	20	12 000	133	90

The symbol (-) means not available. ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, hemoglobin concentration; MCV, mean corpuscular volume; PC, platelet count; WBC, white blood cell count. 6-MMP, 6-methylmercaptopurine; 6-TGN, 6-thioguaninenucleotides (Lennard). Metabolite concentrations are displayed as pmol/8 × 108 red blood cells. All hematologic parameters were determined at the time of metabolite measurement. These values do not have to be the lowest by definition.

White blood cell count differentials

As depicted in **Table 3**, WBC differentials were available in 9/24 patients (38%). In four patients, there was an isolated absolute neutropenia, and in one patient, there was an isolated absolute lymphopenia. In the other four patients, both neutrophil count and lymphocyte count were decreased.

Alternative optimizing treatment strategies after 6-MMPR induced leukopenia

Of all patients developing leukopenia on thiopurine therapy, 11 patients (45%) received subsequent allopurinol 100 mg/day combined with the original thiopurine in a reduced dose (25–33% of original dose), leading to a normalization of 6-MMPR to a median of 220 pmol/8 x 108 RBC [IQR 100-288] ($p<0.01$) in all patients. Concentrations of 6-TGN did neither differ from pre-treatment (6-TGN; median 206 vs 192 pmol/8 x 108 RBC, $p=0.54$) in this subgroup nor in the total group (median 188 vs 193 pmol/8 x 108 RBC, $p=0.95$), but 6-MMPR/6-TGN ratios decreased from a median of 102 to 1.3 ($P < 0.001$) in the total group. In five patients (21%), thiopurine therapy was switched into the alternative thiopurine derivative thioguanine, which undergoes a less complex metabolism without the formation of 6-MMPR. Four patients (17%) with mild myelotoxicity (nos 3, 4, 5, and 16) received a 50% dose reduction of the original thiopurine with subsequent normalization of hematologic parameters. In three patients, 6-MMPR and 6-TGN concentrations decreased (6-TGN to suboptimal levels) after dose reduction, and in the other patients, thiopurine metabolites were not measured. In four patients (17%; nos 5, 6, 11, and 22), thiopurine therapy was discontinued with normalization of hematologic parameters shortly (respectively 21 and 30 days) after discontinuation. Thiopurine therapy was not rechallenged in these patients, based on patient's request. In 20 of 24 (83%) patients, leukocyte count normalized 4 weeks after changing treatment regimen. In the remaining four patients, WBC was not normalized yet but improved compared with the initial leukopenia **(Figure 1)**.

Table 3. Differential effects on white cell line in patients with leukocytopenia and TPMT genotyping (when available)

		Leukocyte differentiation				
CASE	WBC (x109/L)	Neutrophils (× 109/L) [N 1.5–7.5] [N 40–75%]	Lymphocytes (× 109/L) [N 1.0–3.5] [N 25–35%]	Eosinophils (× 109/L) [N < 0.5] [N 0–5%]	Monocytes (× 109/L) [N 0.1–1.0] [N 2–10%]	TPMT genotype
1	2.2	-	-	-	-	-
2	3.7	-	-	-	-	-
3	3.2	1.92 (60%)	0.96 (30%)	0.06 (2%)	0.20 (6%)	-
4	2.7	-	-	-	-	-
5	3.6	-	-	-	-	*1/*1
6	1.7	0.54 (32%)	1.14 (68%)	0.00	0.02 (1%)	-
7	2.3	-	-	-	-	*1/*1
8	2.0	-	-	-	-	-
9	2.5	1.21 (48%)	1.04 (42%)	0.07 (3%)	0.19 (7%)	-
10	3.7	-	-	-	-	*1/*1
11	3.5	1.43 (41%)	1.47 (42%)	0.19 (5%)	0.34 (10%)	-
12	2.6	1.12 (43%)	1.20 (46%)	0.13 (5%)	0.14 (5%)	-
13	3.5	-	-	-	-	-
14	3.9	-	-	-	-	*1/*1
15	1.8	0.64 (36%)	0.97 (54%)	0.07 (5%)	0.09 (5%)	
16	3.8	-	-	-	-	*1/*1
17	3.4	-	-	-	-	-
18	1.8	1.13 (63%)	0.53 (29%)	0.02 (1%)	0.07 (4%)	-
19	2.3	-	-	-	-	-
20	0.9†	0.46 (51%)	0.13 (14%)	0.22 (24%)	0.02 (2%)	-
21	0.8	0.40 (50%)	0.34 (43%)	0.00	0.06 (7%)	-
22	2.2	-	-	-	-	-
23	3.5	-	-	-	-	*1/*1
24	2.5	-	-	-	-	*1/*1

TPMT, thiopurine methyl-S-transferase, WBC, white blood cell count, *1/*1, wildtype genotype.
† 3 days after initial diagnosed leukocytopenia. The symbol (-) means result unavailable. Values expressed in bold are lower than reference values.

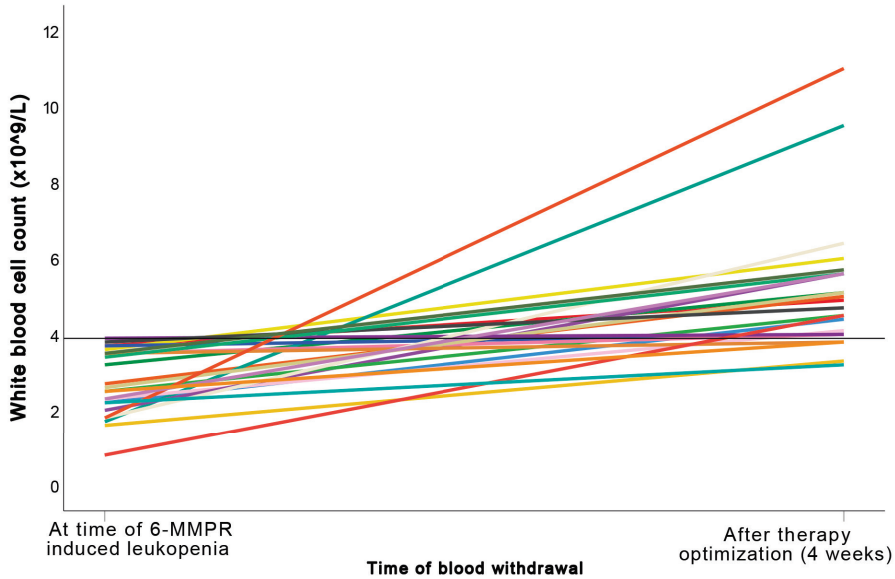

Figure 1. Change in white blood cell count four weeks after optimizing treatment strategy

Anemia at initial presentation resolved in 12/18 (67%) of patients and improved in the remaining six patients. At follow-up, four patients (17%) had thrombocytopenia. No mortality was observed in our cohort. These results are summarized in **Table 4**. Overall, restart with adapted thiopurine therapy was successful in 20 of 24 (83%) patients. 6-MMPR concentrations were neither correlated with the use of co-medication (i.e. mesalazine), nor with the dose of thiopurine therapy in the different patients. The development of leukopenia was not correlated with the use of co-medication. Genotyping of the TPMT enzyme (i.e. wild-type *1/*1 vs. heterozygous/homozygous polymorphisms) was performed using a polymerase chain reaction in seven patients at baseline (29%; nos 2, 5, 7, 10, 16, 23, and 24). All these patients were carriers of the wild-type TPMT genotype (*1/*1).

Table 4. Laboratory parameters of patients developing myelotoxicity on thiopurine therapy due to high 6-methylmercaptopurine concentrations after changing treatment strategy

	After changing treatment strategy					
CASE	WBC (x109/L)	Hb (x109/L)	PC (x109/L)	6-MMPR	6-TGN	Strategy
1	4.4	8.7	264	0	695	Thioguanine
2	4.9	7.2	254	0	140	Thioguanine
3	5.1	9.4	207	2 800	215	Dose reduction
4	5.0	8.4	177	6 800	173	Dose reduction
5	6.0	5.8	226	-	-	Discontinuation
6	9.5	9.1	146	760	0	Discontinuation

Table 4. Continued.

CASE	After changing treatment strategy					
	WBC (x109/L)	Hb (x109/L)	PC (x109/L)	6-MMPR	6-TGN	Strategy
7	4.1	7.1	164	4 500a	68	Thioguanine
8	5.6	9.0	318	310	273	LDTAb
9	4.5	7.6	153	200	207	LDTAb
10	4.0	8.7	264	-	-	Dose reduction
11	4.0	7.3	65	-	-	Discontinuation
12	5.1	7.6	247	280	188	LDTAb
13	3.8	7.9	221	190	219	LDTAb
14	4.0	8.5	228	254	204	LDTAb
15	6.4	7.3	120	-	-	LDTAb
16	4.7	8.0	240	<100	538	LDTAb
17	5.6	7.9	235	0	235	Thioguanine
18	11.9	8.2	395	240	327	LDTAb
19	5.6	-	-	1 300a	27	Thioguanine
20	3.3	8.2	316	<100	162	LDTAb
21	4.5	9.6	229	<100	62	LDTAb
22	3.2	7.4	81	-	-	Discontinuation
23	5.7	8.5	345	880	46	Dose reduction
24	3.8	8.9	183	724	127	LDTAb

Hb, Hemoglobin concentration; PC, platelet count; WBC, white blood cell count; 6-MMP: 6-methylmercaptopurine; 6-TGN: 6-thioguaninenucleotides (Lennard); LDTA, low dose thiopurine and allopurinol. Metabolite concentrations are displayed as pmol/8 × 108 red blood cells. The symbol (-) means not available.
[a]Detectable because mercaptopurine was only terminated recently. [b]addition of allopurinol 100mg/day to a reduced (25–33%) dose of the original thiopurine.

DISCUSSION

In this case series, a detailed description of 24 patients developing myelotoxicity on thiopurine therapy due to a skewed, ultramethylating thiopurine metabolism, and their follow-up is provided. In these patients, 6-MMPR-induced leukopenia developed after a median of 11 weeks after initiation of thiopurine therapy and resolved within 4 weeks upon altered treatment regimen in 83% of the patients. One case has been published before.[13]

Over recent years, metabolism of thiopurines in IBD patients has been extensively investigated. Most dose-dependent adverse events of thiopurines in IBD patients have been ascribed to two metabolite groups, 6-MMPR and 6-TGN. Thiopurine-induced myelotoxicity is almost exclusively being described in relation to grossly elevated 6-TGN levels, causing DNA strand breakage leading to direct cytotoxicity and apoptosis of activated T-lymphocytes. [4,9] High

6-TGN concentrations are associated with low TPMT activity caused by a mutant genotype, thus shifting the balance between 6-MMPR and 6-TGN formation. Besides toxic 6-TGN concentrations, 6-MMPR in (extremely) high concentrations can cause myelotoxicity as well, because of inhibition of de novo purine synthesis.[4,18,24] Purines are essential compounds in nucleic acids, needed for the generation of DNA.[25] When thiopurines are administered in high (oncological) dosages, the median time to develop leukopenia is approximately ten days.[26] In IBD, dosage of thiopurine therapy is substantially lower because of another mode-of-action, because the required effect is mainly antiapoptotic, instead of anti-metabolic.[4] In the current analysis with lower IBD dosages, in which high 6-MMPR concentrations were studied as a result of a skewed thiopurine metabolism, the median time to leukopenia was 11 weeks.[8]

We observed that 11 (45%) patients who developed myelotoxicity because of ultramethylation benefitted from the addition of allopurinol to a reduced (25–33%) dose of the original thiopurine (LDTA). Allopurinol is an inhibitor of the enzyme xanthine oxidase and also has an indirect inhibiting function on TPMT enzyme activity, thus leading to lower 6-MMPR and higher 6-TGN production.[27-29] In addition, allopurinol seems to have an enhancing effect on hypoxanthine-guanine phosphoribosyltransferase (HGPRT), contributing to higher 6-TGN formation as well.[24,30] Additionally, five patients with leukopenia on conventional thiopurine therapy benefitted from a switch to thioguanine therapy, hereby avoiding the formation of 6-MMPR.[31]

As shown in a large prospective cohort study by Chaparro and colleagues, leukopenia was witnessed in about 4% of the patients treated with thiopurines after a median period of 7 months.[32] This finding was underlined in a systematic review by Gisbert et al.[33] This effect is predominantly seen in patients with high 6-TGN concentrations (e.g. caused by heterozygote/homozygote TPMT mutations or patients with a NUDT15 mutation), and incidence is probably lower in patients with high 6-MMPR levels.[34-36]

Recently, results of a prospective study showed that elevated 6-MMPR and 6-TGN metabolites assessed one week after initiation were independently associated with thiopurine-induced leukopenia.[37] Furthermore, it was demonstrated that patients who show excessive 6-MMPR formation are also at risk for early thiopurine failure because of intolerable adverse events or refractoriness.[38]

One of the limitations of our case series is the retrospective nature. Another possible limitation is that patients in this cohort were identified based on a skewed thiopurine metabolism, and these results were linked to leukopenia afterwards. Because therapeutic drug monitoring is not performed routinely in all patients of the participating centers, the total number of 6-MMPR-induced leukopenia might be higher than suggested in our analysis. Unfortunately, differentials of WBC were available in only 9/24 patients, because this measurement is not performed per protocol in the participating centers. Only WBC differentials within 3 days after diagnosed leukopenia were added to our analysis. Interestingly, it is not clear why patients

develop neutropenia and/or lymphopenia, as shown in our results. To our knowledge, there are no data available describing the relative incidence of neutropenia or lymphopenia in thiopurine users.

In this cohort, we did not determine TPMT genotyping systematically. However, we expect all patients to have normal/high TPMT activity (wild-type genotype), because 6-MMPR formation is mainly driven by TPMT activity. Whereas it has been described that preferential 6-MMPR production could occur in patients with TPMT mutations, we believe this will not be of added value to this paper, because other risk factors, besides 6-MMPR, for developing leukopenia are not assessed in this retrospective study.[13] Furthermore, even though we ruled out common causes of leukopenia (e.g. viral infection or sepsis), it is not ruled out that other factors (e.g. hematologic or autoimmune disorders and deficiencies of dietary vitamins) might have contributed to the development of leukopenia in these patients, especially in those patients with only marginal elevated 6-MMPR concentrations.[39]

With this detailed case series, we underline that myelotoxicity may also be caused by grossly elevated levels of 6-MMPR. This is added to what has previously been demonstrated, namely that myelotoxicity is mainly caused by elevated cytotoxic levels of 6-TGN, the use of certain co-medications or intercurrent (viral) infections. Our findings might also be an explanation for unexplained leukopenia during thiopurine therapy without genetic variations (e.g. TPMT or NUDT15 mutation).[25,40,41]

CONCLUSION

We demonstrated that leukopenia develops in patients with (extremely) elevated concentrations of 6-MMPR. Almost all patients were successfully treated with LDTA or from a switch to thioguanine. Adapted thiopurine therapy was successful in the majority of patients who developed leukopenia resulting from a skewed metabolism. As myelotoxicity mainly seems to occur shortly after introduction of thiopurine therapy, we stress the importance of therapeutic drug monitoring in case of myelotoxicity, especially in the first weeks after initiation.

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CHAPTER 4

REAL-LIFE STUDY OF SAFETY WITH THIOPURINE-ALLOPURINOL COMBINATION THERAPY IN INFLAMMATORY BOWEL DISEASE: MYELOTOXICITY AND HEPATOTOXICITY RARELY AFFECT MAINTENANCE TREATMENT

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ABSTRACT

Background: Low-dose thiopurine-allopurinol (LDTA) combination therapy is a commonly applied optimization strategy in IBD patients with a skewed thiopurine metabolism.

Aim: To assess continued LDTA maintenance treatment at annual intervals, and explore risk factors for treatment cessation.

Methods: Adult IBD patients treated with LDTA between 2009-2016 were retrospectively included. Data on the incidence of clinical and laboratory adverse events (AEs), including hepatotoxicity and myelotoxicity, imposing LDTA therapy cessation and associated risk factors were collected.

Results: In total, 221 IBD patients (46% males, median age 42 years) were included. Maintenance LDTA treatment was continued in 78% at 1 year (n=145), 66% at 2 years (n=83), 57% at 3 years (n=52), and 52% at 4 years (n=33). Treatment in patients receiving LDTA therapy for AEs during thiopurine monotherapy was more often continued than in patients initiating LDTA for other indications (i.e. ineffectiveness of thiopurine monotherapy, routinely discovered skewed metabolism) (P=0.016). Myelotoxicity during thiopurine monotherapy resolved in 87% and hepatotoxicity in 86% after median of 1.2 and 1.4 months after LDTA initiation. Cumulative incidence of AEs during LDTA imposing therapy cessation within total follow-up of 449 treatment-years was 7% for clinical AEs, 4% for myelotoxicity and 1% for hepatotoxicity.

Conclusion: LDTA therapy is a safe and beneficial optimization strategy in IBD patients. Continued maintenance LDTA treatment is 52% after 4 years of treatment, and most commonly affected by ineffectiveness of LDTA rather than LDTA-attributed laboratory toxicity. LDTA optimization strategy is most advantageous in patients failing thiopurine monotherapy due to AEs.

INTRODUCTION

Adverse events are an important reason for discontinuation of thiopurine monotherapy in IBD patients. Both clinical adverse events, such as nausea or general malaise, and laboratory adverse events such as hepatotoxicity and leukopenia are common during thiopurine therapy.[1-5] Safety concerns associated with thiopurines can be attenuated by the early identification of toxicity through routine laboratory monitoring and subsequent modification of therapy.[6-8]

Azathioprine (AZA) and mercaptopurine (MP) are converted by three major enzymatic pathways, involving thiopurine methyltransferase (TPMT), into active 6-thioguanine nucleotides (6-TGN) and the byproducts 6-methyl mercaptopurine ribonucleotides (6-MMPR).1 The active 6-TGN metabolites are responsible for the immunosuppressive effect and related clinical response, but high 6-TGN levels are associated with myelotoxicity. The 6-MMPR metabolites are associated with adverse events such as hepatotoxicity and also myelotoxicity.[9, 10] Therapeutic response and toxicity are highly variable between patients, partly explained by differences in formation of these metabolites, due to genetic variants in the genes encoding for crucial enzymes in the thiopurine metabolism.[11]

Therapeutic drug monitoring through assessment of pharmacogenetics and thiopurine metabolite levels, allows for identification of drug metabolism variations, thiopurine under-dosing/ over-dosing, and noncompliance. A considerable proportion of patients harbor drug metabolism variations that lead to a 'skewed thiopurine metabolism', where methylation results in excessive amounts of 6-MMPR at the expense of 6-TGN levels that usually remain below the therapeutic range. These patients are particularly at risk for poor treatment response and development of adverse events.[12] Allopurinol is a xanthine oxidase inhibitor and redirects the thiopurine metabolism towards 6-TGN formation resulting in increased levels of 6-TGN and reduced 6-MMPR levels.[13-15] The optimization strategy of low-dose thiopurine combined with allopurinol (LDTA) improved thiopurine efficacy in patients who previously failed thiopurine monotherapy due to a skewed metabolism. Moreover, liver test abnormalities associated with high 6-MMPR improved upon LDTA therapy.[16-19]

Although LDTA therapy is a commonly applied optimization strategy, data on continued maintenance LDTA treatment and incidence of laboratory toxicity are scarce.[20-22] In this study, we aimed to i) assess continued maintenance LDTA treatment in adult IBD patients and ii) explore the reasons for discontinuation as well as iii) the incidence of laboratory toxicity i.e. hepatotoxicity and myelotoxicity.

METHODS

Study population

A retrospective cohort study was conducted in four tertiary referral centers and two teaching hospitals in the Netherlands. This protocol was approved by the institutional ethical review committee of the corresponding center, and in all participating centers as per local regulations.

Adult IBD patients treated with combination therapy of allopurinol and LDTA, between January 1st 2009 and December 31st 2016 were eligible for this study. Patients were retrieved from the outpatient clinics and local databases, and crosschecked with pharmacy records holding prescriptions for both allopurinol and AZA or MP. Exclusion criteria were unavailability of laboratory assessments, a known history of chronic liver disease (i.e. viral hepatitis, auto-immune hepatitis, steatosis hepatitis, primary sclerosing cholangitis, primary biliary cholangitis or liver cirrhosis), and treatment with concomitant immunosuppressive or immunomodulatory medication, long-term use systemic corticosteroids (i.e. >3 months after start of LDTA therapy), methotrexate, cyclosporin, tacrolimus, mycofenolaatmofetil and/or biological agents), and thiopurine therapy for a non-IBD indication. In addition, patients with short bowel syndrome (<200 cm small bowel) were excluded from analysis, because of possible interference with thiopurine bioavailability as allopurinol is a XO-inhibitor, and XO activity is particularly high in the intestinal mucosa.[23]

Data collection

Individual patient records and laboratory reports were reviewed. Baseline was set at the moment of starting LDTA therapy. Data collection was completed until December 31st 2016. Demographic data were collected including gender, age, weight, diagnosis, smoking behavior and history of IBD related surgery. IBD was classified according to the Montreal Classification for Crohn's disease (CD) or ulcerative colitis/IBD-unclassified (UC/IBDU). Reasons for initiating LDTA therapy were recorded, and categorized into thiopurine monotherapy-attributed AEs (i.e. laboratory toxicity or clinical AEs, ineffectiveness of thiopurine monotherapy, skewed thiopurine metabolism at routinely performed TDM, and allopurinol prescribed for other diseases (e.g. gout). Therapy characteristics were collected including duration of preceding thiopurine monotherapy, type of thiopurine (AZA or MP), dosage of thiopurine and allopurinol, and duration of LDTA therapy.

Continued maintenance LDTA treatment

The primary outcome measure was continued maintenance ongoing LDTA treatment at annual intervals. Secondary outcomes were incidence rates of LDTA-attributed adverse events imposing therapy cessation, and incidence rates of LDTA-attributed laboratory toxicity, i.e. hepatotoxicity and myelotoxicity. Reasons for discontinuation of therapy were explored and subdivided into IBD flare, AEs, long-term remission, and other reasons (e.g. patient initiative and loss to follow-up). Flare of IBD was defined as clinical, biochemical and/or endoscopic

disease activity according to the clinical charts, which led to modification of therapy i.e step-up treatment with systemic corticosteroids and/or immunomodulators or biological agents or surgery. Adverse events during LDTA treatment were divided into clinical adverse events such as fatigue, gastro-intestinal complaints and skin abnormalities, and laboratory toxicity (hepatotoxicity, myelotoxicity).

Evaluation of laboratory toxicity

All laboratory assessments performed during LDTA therapy were recorded, and screened for signs of toxicity. Myelotoxicity was defined as a leukocyte count $<4.0 \times 10^{-9}$/L and/or platelet count $<150 \times 10^{-9}$/L. Leukopenia was classified into mild ($3.0\text{-}4.0 \times 10^{-9}$/L), moderate ($2.0\text{-}3.0 \times 10^{-9}$/L) and severe ($<2.0 \times 10^{-9}$/L). Thrombocytopenia was also classified into mild (100-150 10e9/l), moderate ($50\text{-}100 \times 10^{-9}$/L) and severe ($<50 \times 10^{-9}$/L). When both leukopenia and thrombocytopenia were detected within a laboratory assessment, myelotoxicity was graded based on the most severe value detected. Hepatotoxicity was defined as liver enzymes values above the upper limit of normal (ULN), i.e. alkaline phosphatase, gamma-glutamyltransferase, alanine aminotransferase and/or alanine aminotransferase. Hepatotoxicity was classified into 3 grades according to the Common Terminology Criteria for Adverse Events (CTCAE) v. 3.0.24 Grade 1 is defined as liver tests between ULN and 2.5 x ULN, grade 2 is between 2.5–5.0 x ULN and grade 3 is between 5.0–20.0 x ULN. In line with previous literature, an isolated increase in AP or AST < 2.5 x ULN was not considered as hepatotoxicity as it is not necessarily a sign of liver injury.6, 25, 26 When more than 1 aberrant liver test was detected within a laboratory assessment, hepatotoxicity was graded based on the most severe value detected. In addition, aberrant laboratory values at baseline leading to initiation of LDTA therapy were also collected to evaluate toxicity over time. Dose adjustments due to laboratory adverse events were documented.

Statistical analysis

The primary outcome of this study was continued maintenance LDTA treatment, defined as the percentage of patients on LDTA therapy at 12, 24, 36, 48 and 60 months after initiation of LDTA treatment using Kaplan Meier survival analysis. Treatment cessation was used as the endpoint for analyses regarding continued LDTA treatment, defined as IBD flare, drug withdrawal due to LDTA-attributed AEs (i.e. clinical or laboratory toxicity) or ineffectiveness, cessation of LDTA on patients initiative. All other patients were censored at time of LDTA withdrawal due to long-term remission or last follow-up. Kaplan–Meier curves with separate lines for reasons of initiation of LDTA (i.e. thiopurine monotherapy-attributed AEs, ineffectiveness of monotherapy and optimization of therapy due to TDM) were plotted to explore differences in rates of continued maintenance LDTA treatment. Log-rank tests were used to compare the Kaplan–Meier curves. Univariate Cox proportional hazard models were employed to assess associations between continued maintenance LDTA treatment 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 univariate models, using

stepwise backward elimination (probability of F to remove >0.10). In calculations including drug doses, we calculated the AZA dose into an equivalent MP dose with a conversion factor of 2.08.27 Incidence rates of myelotoxicity and hepatotoxicity were expressed as the percentage of patients with detected laboratory toxicity per patient per treatment year (abbreviated as treatment year) and stratified according to the severity of toxicity (mild, moderate and severe toxicity). Patients were categorized based on the most severe value of myelotoxicity detected during follow-up. Detection rates of myelotoxicity and hepatotoxicity were expressed as the percentage of laboratory assessments showing signs of toxicity. Recovery of baseline laboratory toxicity was defined as the interval between date of commencement of LDTA and date of normalization of laboratory toxicity (date of laboratory assessment). Descriptive data were expressed as numbers with percentages. Continuous variables were expressed as mean with standard deviation (SD) data, or in case of non-normal distributions as median with interquartile range (IQR), defined as the 25th percentile and the 75th percentile. A value of $P<0.05$ was considered to be statistically significant. All analyses were conducted using IBM SPSS Statistics V.21.0 (Armonk, NY, USA: IBM).

RESULTS

Study population characteristics

A total of 221 adult IBD patients (46% males, median age 42 years [IQR 31-54]) treated with LDTA were included, with a total of 449 treatment-years of follow-up (**Table 1**). Hundred forty-three patients (65%) were diagnosed with Crohn's disease (CD) and 78 patients (35%) with UC/IBDU. Reasons for starting LDTA therapy were ineffectiveness of thiopurine monotherapy (45%), skewed thiopurine metabolism at routine TDM (23%), thiopurine monotherapy-attributed laboratory toxicity i.e. hepatotoxicity and/or myelotoxicity (12%), clinical adverse events (3%) and combined ineffectiveness and laboratory toxicity (10%) (**Table 1**). In total, 133 patients (60%) were treated with LDTA and AZA (adjusted median dose 0.3 mg/kg [IQR 0.3-0.4]) and 88 patients (40%) were treated with LDTA and MP (median dose 0.4 mg/kg [IQR 0.3-0.5]). No statistically significant differences were observed in dosages between AZA and MP (P=0.370). Co-prescription of 5-ASA was used as maintenance strategy in 48 patients (22%). Corticosteroids for induction treatment at the initiation of LDTA therapy was recorded in 34 patients (15%).

Table 1. Baseline cohort characteristics

	N = 221
Male sex, n (%)	101 (46)
Age (yr)	42 .8 [31.4-55.0]
Age at diagnosis (yr)	30.8 [21.9-44.0]
Weight (kg)[a]	76 [66-87]
Diagnosis, n (%) CD UC/IBDU	143 (65) 78 (35)
CD – Montreal age at diagnosis, n (%) A1 <17 yr A2 17-40 yr A3 >40 yr	 14 (10) 96 (67) 33 (23)
CD – Montreal localization, n (%) L1 Ileal L2 Colonic L3 Ileocolonic L4 Upper GI disease Perianal involvement	 60 (42) 29 (20) 54 (38) 7 (5) 27 (19)
CD – Montreal disease behavior B1 Non-stricturing, non-penetrating B2 Stricturing B3 Penetrating	 80 (56) 38 (27) 25 (17)
UC – Montreal disease extent[b] E1 Proctitis E2 Left-sided E3 Extensive colitis	 6 (8) 28 (36) 44 (56)
Bowel surgery, n (%)	42 (19)
Current smokers, n (%)[a]	50 (23)
Median duration thiopurine monotherapy, months [IQR]	7.5 [2.1-36.6]
LDTA therapy AZA + allopurinol MP + allopurinol	133 (60) 88 (40)
Thiopurine dose (mg/kg)[a] AZA MP Allopurinol dose (mg)	0.3 [0.3-0.4] 0.4 [0.3-0.5] 100 [100-100]
Concomitant IBD medication, n (%) 5-ASA Steroids during induction	48 (22) 34 (15)
Reason initiation LDTA, n (%) Ineffectiveness monotherapy Adverse events thiopurine monotherapy Laboratory toxicity Clinical adverse events Ineffectiveness + laboratory toxicity Routinely discovered skewed metabolism Other	100 (45) 26 (12) 7 (3) 23 (10) 50 (23) 15 (7)

IBD, inflammatory bowel disease; Yr, years; IQR, interquartile range; kg, kilogram; UC, ulcerative colitis; CD, Crohn's Disease; IBDU, IBD-unclassified; AZA, azathioprine; MP, mercaptopurine; mg, milligram; 5-ASA, 5-Aminosalicylic acid; TPMT, thiopurine S-methyltransferase. Data are reported as median with interquartile range.
a Missing data for weight, n=1; for smoking status n=36; for thiopurine dose, n= 1; b Combined for UC and IBDU.

Continued maintenance LDTA treatment

Maintenance LDTA treatment in IBD patients was continued in 78% at 1 year (n=145), 66% at 2 years (n=83), 57% at 3 years (n=52), and 52% at 4 years (n=33) (**Figure 1**). After a median follow-up of 19.4 months [IQR 8.5-34.9], treatment was discontinued in 94/221 patients (43%) (**Table 2**).

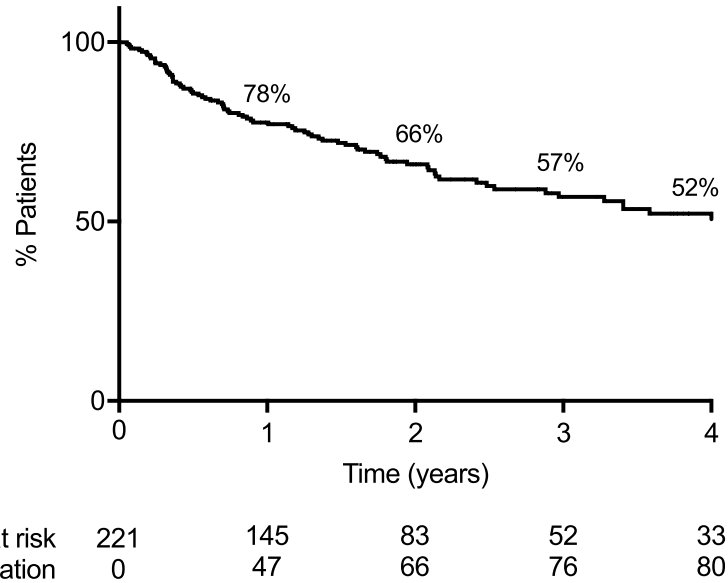

Figure 1. Continued LDTA maintenance treatment. Kaplan-Meier estimates of time to drug cessation after LDTA onset (T0) are shown. Patients were censored at time of LDTA withdrawal due to long-term remission or last follow-up.

Reasons for withdrawal of LDTA were ineffectiveness (15%), LDTA-attributed AEs 12%), on patient's initiative (9%), long-term remission (3%) and other reasons (4%). With regard to the AEs imposing LDTA cessation, 15 patients (7%) stopped treatment due to clinical AEs (details in **Table 2**), 9 patients (4%) for myelotoxicity and 4 patients (2%) for hepatotoxicity. A significantly greater proportion of patients treated with MP stopped LDTA treatment during follow-up than AZA users (47.7% vs. 29.3%, P=0.005). In subgroup analysis of patients that withdrew LDTA, thiopurine dose was higher in MP patients than in AZA patients (0.42 mg/kg (SD 0.17) vs. 0.35mg/kg (SD 0.12), P=0.030)). No difference was observed in the rate of continued LDTA maintenance therapy in patients with concomitant 5-ASA therapy (P=0.985).

Table 2. Clinical outcome of LDTA therapy

	N = 221
Cessation of LDTA therapy, n (%)	94 (43)
Reason for cessation, n (%)	
Ineffectiveness	34 (15)
Remission	7 (3)
On patient initiative	16 (9)
Family planning	2 (1)
Adverse events	
Clinical AEs[a]	
Skin abnormalities	5 (2)
Alopecia	2 (1)
General malaise, fatigue	5 (2)
Infections[b]	1 (0.5)
Other	2 (1)
Laboratory toxicity	
Myelotoxicity[c,d]	9 (4)
Hepatotoxicity[c]	2 (1)
Lost to follow-up	6 (3)
Other/unknown	3 (1)

Reasons for cessation of LDTA therapy. AEs, adverse events; IQR, interquartile range. [a] The reported LDTA-attributed adverse events imposed therapy cessation. [b] Patient discontinued due to recurrent viral (airway) infections, no laboratory toxicity was observed. [c] Myelotoxicity was defined as a leukocyte count $<4.0 \times 10^{-9}$/L and/or platelet count $<150 \times 10^{-9}$/L. Hepatotoxicity was defined as liver enzymes values above the upper limit of normal (ULN), i.e. alkaline phosphatase (AP), gamma-glutamyltransferase (ɣ-GT), alanine aminotransferase (ALT) and/or alanine aminotransferase (AST). Both myelotoxicity and hepatotoxicity were classified into 3 grades according to the Common Terminology Criteria for Adverse Events (CTCAE) v. 4.0. [d] During follow-up, 8 dose reductions were performed due to leukopenia.

Overall, continued LDTA maintenance treatment in patients receiving therapy for monotherapy-attributed AEs was 77% after median follow-up of 22 months [IQR 10-40] and significantly higher than 56% in the total group of patients initiating LDTA for other reasons after median follow-up of 19 months [IQR 8-34] (P=0.016) (**Figure 2**, log-rank P=0.03).

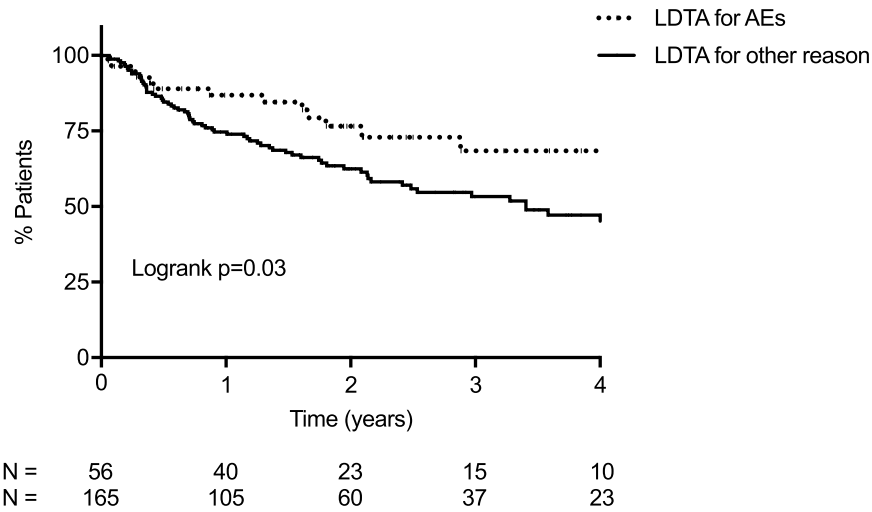

Figure 2. Continued LDTA maintenance treatment related to reason for initiation. The dotted line represents continued treatment in patients that initiated LDTA therapy for AEs during thiopurine monotherapy (n=56). The black line represents continued treatment in patients that initiated LDTA therapy for other reasons than AEs i.e. Ineffectiveness monotherapy, routinely discovered skewed metabolism or other reasons (n=165).

In univariate cox regression analysis, LDTA initiated for AEs was associated with longer LDTA maintenance treatment (Hazard Ratio (HR) 1.38 (95% CI 1.03-1.86, P=0.034)). Also, a higher thiopurine dose was associated with longer LDTA maintenance treatment (HR 1.73 (95% CI 0.41-7.27, P=0.055)). However, in multivariable Cox regression analysis no statistically significant association was observed. The presence of myelotoxicity (HR 1.52, P=0.082) and LDTA initiated for monotherapy-attributed AEs during monotherapy (HR 1.77, P=0.063) showed no statistically significant difference in longer LDTA maintenance treatment (**Table 3**). No other factors, including diagnosis, gender, age or disease characteristics, were associated with continued LDTA maintenance treatment.

Table 3. Covariates associated with continued maintenance LDTA treatment

	Univariate analysis		Multivariable analysis	
	Hazard ratio (95% CI)	**P-value**	**Hazard ratio (95% CI)**	**P-value**
Male gender	0.80 (0.52-1.23)	0.308		
UC/IBDU[a]	1.00 (0.64-1.57)	0.997		
Age at baseline (years)	0.99 (0.97-1.00)	0.153*	0.99 (0.98-1.01)	0.475
Active smoker	0.909 (0.48-1.69)	0.740		
Azathioprine[b]	1.33 (0.86-2.07)	0.198*	0.67 (0.42-1.07)	0.093
Thiopurine dose[c]	1.73 (0.41-7.27)	0.055*	1.83 (0.41-8.19)	0.428
LDTA initiated for AEs[d,e]	1.38 (1.03-1.86)	0.034*	1.77 (0.97-3.25)	0.063

UC, Ulcerative colitis; IBDU, IBD-unclassified; AEs, adverse events; CI, confidence interval; HR, hazard ratio; LDTA, low dose thiopurine and allopurinol. * Covariates with a p-value <0.2 were included in the multivariable regression analysis. [a] IBD diagnosis, Ulcerative colitis/IBD-unclassified vs. Crohn's disease. [b] Drug type, mercaptopurine vs. Azathioprine. [c] Azathioprine dose in mg/kg was divided by a factor 2.08 to compare with MP dosage. [d] LDTA initiated for thiopurine monotherapy-attributed AEs (n=56) compared to the total group of patients initiating LDTA for other reasons (n=165). [e] Adverse events comprise both clinical AEs and laboratory toxicity. Presence of toxicity (hepatotoxicity and/or myelotoxicity) was classified according to the Common Terminology Criteria for Adverse Events (CTCAE) v. 4.0

Laboratory toxicity

During LDTA treatment, 130 patients (59%) had signs of toxicity in one or more laboratory assessments (**Table 4**). Signs of myelotoxicity and/or hepatotoxicity were detected in 481/1827 assessments (26%). Thiopurine dose was slightly higher in patients with laboratory toxicity during LDTA than in patients without laboratory toxicity (0.40 mg/kg (SD 0.16) vs. 0.36 mg/kg (SD 0.13)), without statistical significance (P=0.053). No difference was observed in laboratory toxicity between patients with or without concomitant treatment with 5-ASA (P=0.297). Myelotoxicity was observed in 87/221 (39%) patients, and in 265/1827 assessments (15%). In addition to 7% of patients (n=15) that showed myelotoxicity at baseline, the incidence rate of myelotoxicity was 39% (n=64) during the first year of LDTA treatment. After the first year, myelotoxicity incidence rate was 11% per treatment year. The majority of detected myelotoxicity in laboratory assessments was classified as mild (86%) and comprised leukopenia in 94%. No cases of severe myelotoxicity were detected in this cohort. LDTA therapy was stopped in 9 patients (4%) due to de novo myelotoxicity (mild n=4, moderate n=5). In addition, myelotoxicity was managed by thiopurine dose reduction in 8 patients (4%). All patients were then able to continue LDTA therapy after thiopurine dose reduction. Median follow up after dose reduction was 13.6 months (IQR 6.7-27.7). Ongoing LDTA use was documented in 7 patients until the end of follow up and 1 patient stopped LDTA treatment due to sustained remission.

Table 4. Detection rate of laboratory toxicity

	Patients	**Overall assessments**
	n=221	**n= 1827**
Laboratory toxicity, n (%)	130 (58.9)	481 (26.3)
Myelotoxicity, n (%)	87 (39.4)	265 (14.5)
Mild	62 (28.0)	229 (12.5)
Moderate	25 (11.3)	36 (2.0)
Severe	-	-
Hepatotoxicity, n (%)	68 (30.1)	259 (14.2)
Mild	50 (22.6)	236 (12.9)
Moderate	9 (4.1)	13 (0.7)
Severe	9 (4.1)	10 (0.5)

Incidence rate of toxicity during LDTA therapy. Myelotoxicity was defined as a leukocyte count <4.0 x 10^{-9}/L and/or platelet count <150 × 10^{-9}/L.. Hepatotoxicity was defined as liver enzymes values above the upper limit of normal (ULN), i.e. alkaline phosphatase (AP), gamma-glutamyltransferase (γ-GT), alanine aminotransferase (ALT) and/or alanine aminotransferase (AST). Both myelotoxicity and hepatotoxicity were classified into 3 grades according to the Common Terminology Criteria for Adverse Events (CTCAE) v. 4.0. Patients were classified according to the most severe grade of toxicity. Combined myelotoxicity and hepatotoxicity was detected in 27 patients (12%).

Hepatotoxicity was present in 259/1827 (14%) assessments in 69/221 patients (31%). In addition to 17% of patients (n=37) that initiated LDTA due to monotherapy-attributed hepatotoxicity during thiopurine monotherapy with signs of hepatotoxicity present at baseline, the incidence rate of hepatotoxicity in the first year of LDTA treatment was 22% (n=36). The incidence rate of hepatotoxicity after the first year was 11% per treatment year. The majority of detected hepatotoxicity was classified as mild (91%), 5% moderate and 4% severe. Two patients (1%) with de novo hepatotoxicity had to withdraw LDTA. Clinical treatment-related complications with concurrent laboratory toxicity were detected in 2 patients (10.9%). Both patients were admitted to the hospital and received a transfusion with packed cells for anemia (n=1) or pancytopenia (n=1). No cases of nodular regenerative hyperplasia were reported. No mortality was observed.

Laboratory toxicity prior to LDTA

Within the subgroup of 49 patients that started LDTA due to thiopurine monotherapy-attributed laboratory toxicity, myelotoxicity resolved in 10/12 patients (83%), hepatotoxicity resolved in 29/34 patients (85%) and laboratory abnormalities normalized in all 3 patients with combined toxicity (100%). Median time to myelotoxicity resolved after a median duration of 1.2 months [IQR 0.8-5.4] and hepatotoxicity after 1.4 months [IQR 0.7-5.0] (**Figure 3**). None of these 50 patients had to withdraw LDTA due to laboratory toxicity.

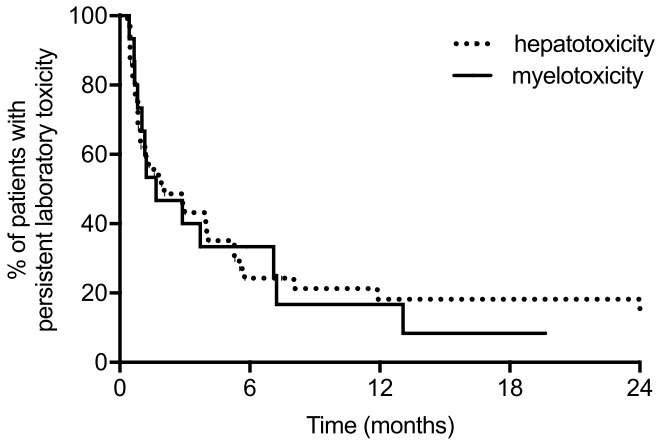

Figure 3. Resolution of thiopurine-monotherapy associated laboratory toxicity during LDTA therapy. The black line represents time to normalization of myelotoxicity established with laboratory assessment obtained during LDTA therapy (n=15). The dotted line represents time to normalization of hepatotoxicity established with laboratory assessment obtained during LDTA therapy (n=37).

DISCUSSION

In this large real life cohort of 221 of IBD patients maintenance LDTA treatment was continued in 78% after 1 year, 66% after 2 years, 57% after 3 years, and 52% at 4 years of treatment. Although mild myelotoxicity and hepatotoxicity are commonly detected during LDTA, these laboratory results rarely induce LDTA withdrawal. Our data demonstrate that LDTA optimization strategy is beneficial in IBD, especially in patients failing thiopurine monotherapy due to AEs.

In this study, we confirm that long-term continued maintenance LDTA treatment is considerable and tolerance of LDTA in patients after failure of thiopurine monotherapy. As definitions for effectiveness of LDTA vary among available studies, direct comparison of the results is difficult. However, considerably high effectiveness was reported uniformly, regardless of the outcome measure after failure of thiopurine monotherapy. Studies evaluating effectiveness of LDTA using clinical disease activity scores have reported steroid-free clinical response, i.e. decline in Harvey Bradshaw Index or Simple Clinical Colitis Activity Index ≥3, ranging from 65% after median follow-up of 19 months to 86% after median follow-up of 31 months.[19, 28]A retrospective trial reported clinical remission (Harvey Bradshaw Inde ≤3 or Lichtiger Index ≤3) in 64% of LDTA treated patients an average of 34 months.[28] Data on endoscopic effectiveness of LDTA are scarce, and remarkably only data in patients with clinical response to LDTA for the total group of both thiopurine naïve patients and after monotherapy failure have been reported: endoscopic improvement was observed in 92% of the patients and endoscopic remission in 54% of patients after median follow-up of 19 months.[19] Similar to the indicator of effectiveness of LDTA in our study, Hoentjen et al. evaluated long-term drug survival of LDTA at yearly time

points in 77 IBD patients failing thiopurine monotherapy.[21] Reported drug survival rates in this smaller cohort of patients were slightly higher compared to our cohort: 87% at 1 year, and 65% at 5 years of follow-up. A possible explanation for these differences is that 9% of patients in this cohort received co-treatment with anti-TNF during LDTA therapy, possibly enhancing a more favorable outcome.[29] In addition, a disease flare during LDTA therapy demanding (temporary) addition of systemic corticosteroid treatment was considered as cessation of LDTA therapy, whereas in the cohort of Hoentjen et al. rescue treatment was not censored in the survival analysis.[21, 30 14]

In this study, we have shown that LDTA is particularly beneficial in patients failing monotherapy due to AEs, including laboratory toxicity. The following three observations substantiate this conclusion. Firstly, we have shown that overall continued LDTA maintenance treatment is higher in patients starting LDTA for thiopurine monotherapy-attributed AEs, than in patients starting LDTA for other indications, including ineffectiveness of monotherapy or patients with a skewed thiopurine metabolism at routinely performed TDM. Secondly, we have shown that mild hepatotoxicity and myelotoxicity are detected often during LDTA, however severe laboratory toxicity is uncommon. Thirdly, our study showed that thiopurine monotherapy-associated myelotoxicity and hepatotoxicity frequently improved after LDTA initiation and none of the patients with laboratory AEs as indication for LDTA stopped treatment for laboratory toxicity.

We observed high rates of continued maintenance treatment in patients starting LDTA after failing monotherapy thiopurines due to AEs. However, these observations are not in line with the results of a recent cohort study by Pavlidis et al.[19] The latter study showed that overall clinical response rates of LDTA (Harvey Bradshaw Index and Simple Clinical Colitis Activity Index scores) were comparable in a subgroup of patients failing monotherapy due to AEs (n=74) and the subgroup of patients that received LDTA for ineffectiveness of monotherapy (n=116) (61% vs. 65%). In addition to a different outcome measure for therapy effectiveness used in both studies, 92% of patients used concomitant steroids at start of LDTA in the study of Pavlidis et al., as compared to only 15% in our study. The high percentage of symptomatic patients in the subpopulation of patients with monotherapy-attributed AEs as the primary indication for LDTA in the study from Pavlidis et al. probably explains the difference between the study findings, as therapy effectiveness will be different between symptomatic and asymptomatic patients with laboratory toxicity.

Although patients remain susceptible to mild laboratory toxicity during LDTA therapy, no cases of severe myelotoxicity were observed and severe hepatotoxicity was detected rarely in our cohort. We observed that the incidence rate of myelotoxicity is highest in the first year of treatment (39%), and decreased to 11% per year afterwards. These data can only be compared to other reports with cumulative incidences, as yearly incidence rates of myelotoxicity LDTA are unavailable. Myelotoxicity was present in 39% of patients during median follow-up of 19.4 months. This incidence is within the wide range of reported incidences of myelotoxicity in

LDTA, varying from 1.3% within median follow-up of 19 months to 48% within median follow-up of 15 months.[16, 19-21, 28, 30] The wide range of incidences of myelotoxicity results from varying definitions, and the high percentage of mild myelotoxicity, illustrated by the observation in our study that 71% of patients with myelotoxicity had WBC between 3.0 and 4.0 × 10^{-9}/L. In our study, a conservative definition of myelotoxicity was used, to avoid underestimation of clinical consequences of laboratory toxicity. As a result, the incidence of myelotoxicity may be somewhat overestimated. Another possible explanation for the wide range of reported rates of myelotoxicity in literature is the use of either AZA or MP.[19, 28] Mercaptopurine has been associated with dose-dependent side effects, in particular myelotoxicity. [28, 31] This finding has been attributed to higher dosing of MP, due to limited availability of pharmaceutical dosage forms of MP. Our data do not confirm higher dosing of MP than AZA in LDTA therapy. Similar to myelotoxicity, we found that the incidence rate of hepatotoxicity was as high as 22% in the first year of treatment, and decreased to 11% per patient year during maintenance treatment. These data add significantly to available literature as data are currently lacking in available reports.

Another benefit of LDTA treatment is the rapid recovery of baseline thiopurine monotherapy-attributed laboratory toxicity after LDTA initiation.[19, 21, 28, 32, 33] We observed high success rates in the resolution of hepatotoxicity in 86% of LDTA treated patients. Remarkably, we also observed benefit of LDTA prescribed for thiopurine therapy associated myelotoxicity in 87% of these patients. Indeed, it has been described that myelotoxicity can be induced by the presence of substantially elevated 6-MMPR levels.[9] Allopurinol is an inhibitor of xantine oxidase and has an indirect inhibiting function on TPMT enzyme activity, and therefore induces a shift in metabolites favouring 6-TGN and lowering 6-MMPR production.[32, 34] In addition, allopurinol seems to have an enhancing effect on hypoxanthine-guanine phosphoribosyltransferase (HGPRT), contributing to higher 6-TGN formation as well.[13, 15, 35] This therapeutic profile is associated with low AE rates and high efficacy. The prompt normalization of monotherapy-attributed myelotoxicity after initiation of LDTA observed in this cohort supports this hypothesis. An incidental finding, that underlines the insignificant role of laboratory toxicity during LDTA, was that the presence of hepatotoxicity was a predictor of longer LDTA treatment (HR 1.74, data not shown), even when excluding patients that initiated LDTA due to thiopurine monotherapy-attributed hepatotoxicity in post-hoc analysis (HR 2.53). Both higher rates of continued LDTA maintenance treatment as well as the presence of this dose-dependent AE could be explained by a higher thiopurine dose established in patients with laboratory toxicity, probably by increased efficacy.

Our study describes a large real-world IBD population on LDTA after thiopurine monotherapy, with detailed information on an individual patient level, especially regarding laboratory toxicity. A major strength of this study is that it reflects current clinical practice in a real-life, non-standardized cohort, which enables the generalizability of these results. Nevertheless, some limitations associated with this retrospective study design need to be addressed. First, due to the retrospective study design, we are not able to draw conclusions on long-term

remission rates during LDTA as data on endoscopic parameters and faecal biomarkers were not collected. Second, it has to be noted that the decision for LDTA commencement was not always guided by 6-TGN and 6-MMPR measurements. Therefore, it is not possible to distinguish between ineffectiveness of thiopurine monotherapy due to a skewed metabolism or non-response to thiopurine therapy in general, possibly affecting susceptibility to LDTA therapy. In addition, considerations regarding favourable thiopurine dose in LDTA therapy is hampered by the lack of available thiopurine metabolites during LDTA treatment. Nevertheless, our data show that LDTA combination therapy can be safely used in clinical practice, even when thiopurine metabolite measurements are unavailable. Third, a majority of patients (78%) was selected from tertiary referral hospitals. Rates of LDTA ineffectiveness might have been increased in these patients due to the complexity of IBD disease course. Also, this might have led to more frequent outpatient visits and laboratory assessments during LDTA therapy and thus a higher detection rate of toxicity and clinical AEs interfering with therapy outcome

In conclusion, LDTA therapy is a safe and beneficial optimization strategy in IBD patients and should not be disregarded after failure of thiopurine monotherapy. A considerably high rate of continued LDTA maintenance treatment during was established during long-term follow-up and seems most advantageous in patients failing thiopurine monotherapy due to adverse events. Although mild myelotoxicity and hepatotoxicity are commonly observed, they rarely affect LDTA treatment cessation.

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CHAPTER 5

LIMITED ADDED VALUE OF LABORATORY MONITORING IN THIOPURINE MAINTENANCE MONOTHERAPY IN INFLAMMATORY BOWEL DISEASE PATIENTS

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On behalf of the initiative on Crohn and Colitis (ICC)

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ABSTRACT

Background: To timely detect myelotoxicity and hepatotoxicity, laboratory-monitoring at 3-month intervals is advised throughout thiopurine maintenance treatment for inflammatory bowel disease (IBD). However, reported incidence rates of myelotoxicity and hepatotoxicity in maintenance treatment are low.

Aim: To assess incidence rates and clinical consequences of myelotoxicity and hepatotoxicity in thiopurine maintenance therapy after at least 1 year of thiopurine treatment.

Methods: Retrospective analysis of therapy adjustment for laboratory toxicity in adult IBD patients after 12 consecutive months of azathioprine (AZA) or mercaptopurine monotherapy (i.e. baseline) between 2000-2016. Incidence rates of laboratory toxicity (i.e. myelotoxicity (leukocyte count<4.0x10e9/L, and/or platelet count<150x10e9/L) and/or hepatotoxicity (AP, GGT, ALT, and/or AST above ULN, excluding isolated increased AST/AF)) and associated diagnostic procedures and complications were assessed.

Results: In total, 12.391 laboratory assessments were performed in 1132 patients (56% female, AZA 74%) during 3.3 years median follow-up. Median monitoring frequency was 3.1 assessments/treatment-year. Only 83/12.391 (0.7%) assessments resulted in therapy adjustment, dose-reduction in 46 patients, cessation in 28 and allopurinol initiation in 9; risk of therapy adjustment was 1.9%/treatment-year. Incidence rates of myelotoxicity were 7.1% (5.1% mild/1.8% moderate/0.1% severe) and hepatotoxicity 5.1% (3.8% mild/1.1% moderate/0.2% severe) per treatment-year. Treatment-related complications with concurrent laboratory toxicity occurred in 12 patients (1.1%) and would not have been prevented by monitoring.

Conclusion: Severe laboratory toxicity is uncommon after 1 year of thiopurine monotherapy at 4-month monitoring intervals. Therapy adjustments are rare after detection of laboratory toxicity. After 1 year of thiopurine monotherapy, laboratory-monitoring may be lowered to less than a 4-month interval.

INTRODUCTION

Thiopurines, azathioprine (AZA) and mercaptopurine (MP), are an effective maintenance therapy for patients with inflammatory bowel disease (IBD). Advantages of thiopurine therapy include a steroid-sparing effect, and its association with a reduced risk of colorectal carcinoma.[1-3] A recent study on the effect of thiopurines on the natural history of ulcerative colitis showed that thiopurine continuation was associated with a lower rate of hospital admission and a reduced risk of progression of disease extent and a colectomy.[1] Adverse events (AEs) are the most important downside of thiopurine therapy and result in therapy withdrawal in up to 40% of patients, primarily within the first months of treatment.[4-6] These AEs can be divided into dose-independent events, such as pancreatitis and arthralgia, and dose-dependent events. The most alarming dose-dependent AEs, hepatotoxicity and myelotoxicity, warrant laboratory monitoring, including a full blood count (FBC) and serum liver enzyme tests (LTs).[7] The risk of laboratory toxicity is high in the first year of treatment, which is reflected by high incidence rates of 11% for myelotoxicity and 13% for hepatotoxicity.[8-10] Myelotoxicity and hepatotoxicity rates may be reduced after the introduction of thiopurine S-methyltransferase (TPMT) genotype testing before- and/or assessment of drug metabolites in thiopurine therapy.[7,11]

Safety concerns associated with thiopurines can be attenuated by the early identification of toxicity through routine laboratory monitoring and subsequent modification of therapy.[8,10-13] This potential benefit of laboratory monitoring should be balanced against the burden for patients and associated direct and indirect healthcare costs. Currently, international guidelines advise an intensive laboratory monitoring schedule in the first 3 months of treatment.[14] During subsequent maintenance therapy, routine laboratory monitoring at 2 to 3 month intervals is recommended. Laboratory toxicity is not always clinically relevant as it often reverses spontaneously.[9,10,15,16] Also, laboratory toxicity usually develops within the first few months of treatment and the reported incidence rate in maintenance treatment is low.[9,10] In addition, leukopenia can develop at any time during treatment without preceding signs of myelotoxicity.[9,15] Therefore, frequent routine assessment of laboratory parameters in long-term maintenance thiopurine therapy may have a limited clinical impact. Data on therapy adjustments or diagnostic procedures based on toxicity found with laboratory monitoring are lacking. This study aims to assess the incidence rate and clinical consequences of myelotoxicity and hepatotoxicity detected with the current laboratory monitoring regimen in IBD patients who have been on thiopurine maintenance therapy for more than 1 year.

METHODS

Study design and patient selection

A retrospective cohort study was conducted in four tertiary referral centers and two teaching hospitals in the Netherlands. Adult IBD patients with confirmed diagnosis of Crohn's disease

(CD), ulcerative colitis (UC), or IBD-unclassified (IBDU), treated with AZA or MP between January 1st 2000 and December 31st 2016 were included after 1 year of thiopurine treatment. Inclusion criteria were maintenance thiopurine monotherapy, defined as 12 consecutive months of treatment, and quiescent disease, defined as clinical, systemic steroid-free remission without the need for step-up treatment. Exclusion criteria were unavailability of laboratory assessments, a known history of chronic liver disease (i.e. viral hepatitis, auto-immune hepatitis, steatosis, primary sclerosing cholangitis, primary biliary cholangitis or liver cirrhosis), treatment with concomitant immunosuppressive medication (systemic corticosteroids, methotrexate, cyclosporin, tacrolimus, mycophenolate mofetil and/or biological agents), and thiopurine therapy for a non-IBD indication. In addition, patients with short bowel syndrome (<200 cm small bowel) were excluded from analysis, because of possible interference with thiopurine absorption. This study conformed to the principles of the Declaration of Helsinki and was approved by the institutional ethical review committee of the corresponding center and all participating centers as per local regulations.

Data collection

Baseline was set at the first laboratory assessment after 1 year of thiopurine treatment. Data collection was completed December 31st 2016. The following patient characteristics were collected from the patient's electronic medical records: gender, weight, smoking status, and IBD-type and Montreal classification. Treatment characteristics included type of thiopurine, date of initiation, dosage, concomitant IBD medication, and TPMT genotype. Laboratory results performed throughout maintenance thiopurine treatment (i.e. after 1 year of treatment) were recorded and screened for toxicity. Myelotoxicity was defined as leukopenia and/or thrombocytopenia. Leukopenia was classified into mild (3.0-4.0x10e9/L), moderate (2.0-3.0x10e9/L) and severe (<2.0x10e9/L). Thrombocytopenia was also classified into mild (100-150x10e9/L), moderate (50-100x10e9/L) and severe (<50x10e9/L). When both leukopenia and thrombocytopenia were detected within a laboratory assessment, myelotoxicity was graded based on the most severe detected value. Hepatotoxicity was defined as abnormal liver tests (LTs), i.e. alkaline phosphatase (AP), gamma-glutamyltransferase (ɣ-GT), alanine aminotransferase (ALT) and/or alanine aminotransferase (AST), defined as an increase above the upper limit of normal (ULN). Hepatotoxicity was classified into 3 grades according to the Common Terminology Criteria for Adverse Events (CTCAE) v. 4.0. [17] Grade 1 is defined as LTs between ULN-2.5xULN, grade 2 is between 2.5–5.0xULN and grade 3 is between 5.0–20.0xULN. In line with previous literature, an isolated increase in AP or AST < 2.5xULN was not considered as hepatotoxicity as it is not necessarily a sign of liver injury.[10,18] When more than 1 aberrant LT was detected within a laboratory assessment, the grade of hepatotoxicity was based on the most severe detected value. When myelotoxicity and hepatotoxicity were detected in 1 assessment, the severity of 'combined' toxicity was based on the most severe detected grade. Treatment changes and/or additional diagnostic procedures were recorded for patients with laboratory toxicity. Patients were followed until treatment cessation or end of follow up at December 31st 2016. Reasons for cessation of treatment included disease flare warranting

step-up treatment or initiation of therapy with systemic (gluco-cortico)steroids, long-term remission, AEs, on patient initiative, family planning or loss to follow up.

Outcomes

The primary outcome was therapy adjustment based on laboratory toxicity, defined as therapy cessation, dose reduction or additional therapy with allopurinol alongside a reduced thiopurine dose (LDTA). Secondary outcomes were additional diagnostic procedures triggered by laboratory toxicity, incidence rates of myelotoxicity and hepatotoxicity and laboratory toxicity related complications. Additional diagnostic procedures comprised extra laboratory assessments, abdominal ultrasonography, magnetic resonance imaging, bone marrow examination and liver biopsy. Complications associated with concurrent laboratory toxicity (including hospitalization, surgery or infections) were classified according to the CTCAE (version 4.0) and categorized according to system organ class and severity. Severity was subdivided in grade 1 (asymptomatic or mild symptoms without indication for treatment or intervention), grade 2 (moderate symptoms; local or non-invasive intervention indicated), grade 3 (severe medically significant symptoms, invasive treatment and/or hospitalization indicated), grade 4 (life-threatening consequences) or grade 5 (death related to AE). Only therapy-related clinical complications were included in the analysis.

Statistics

The primary outcome of this study was defined as therapy adjustments based on laboratory toxicity. For Kaplan-Meier survival analyses, patients without therapy adjustments were censored at time of last follow-up or treatment cessation for other reasons than laboratory toxicity. Characteristics between patients with and without AEs were compared by the chi-square test for dichotomous variables, and student's t tests or Mann–Whitney U tests were used for continuous variables. Univariate and multivariable Cox proportional hazards models and logistic regression models were performed to assess risk factors of patient characteristics and laboratory covariates associated with therapy adjustments, and the time of development of laboratory toxicity. Variables with a $P<0.20$ were included in a multivariate Cox-proportional hazard model. Incidence rates of myelotoxicity and hepatotoxicity were expressed as the percentage of patients with detected laboratory toxicity per patient per treatment year (abbreviated as treatment year). Cumulative incidence of myelotoxicity and hepatotoxicity was calculated using Kaplan-Meier estimates, and stratified according to the most severe value (mild, moderate or severe toxicity), with time to event set at the first event in the corresponding category of severity. Detection rates of myelotoxicity and hepatotoxicity were expressed as the percentage of laboratory assessments showing signs of toxicity. The AZA drug dose was calculated into an equivalent pharmaceutical MP dose with a conversion factor of 2.08 based on molecular weight and bioavailability, the so-called AZA adjusted dose.[19] Data are presented as median and its interquartile range for continuous variables when applicable. A value of $P<0.05$ was considered to be statistically significant. All analyses were conducted using IBM SPSS Statistics V.22.0 (Armonk, NY, USA: IMB).

RESULTS

Cohort characteristics

A total of 1132 IBD patients on long-term thiopurine treatment were included (56% female, median age 37 years (IQR 26-49). In total, 843 patients (74%) were treated with AZA (adjusted median dose 0.9 mg/kg (IQR 0.8-1.0)) and 289 patients (26%) were treated with MP (median dose 0.8 mg/kg [IQR 0.6-1.1]). Median follow up until cessation of therapy or censoring was 3.3 years [IQR 1.7-5.6] (**Table 1**). Treatment was discontinued in 641 patients (57%) after median follow up of 4.4 years [IQR 28-6.7]. Reasons for discontinuation were IBD flare in 265 patients (23%), sustained remission in 167 patients (15%), on patient initiative in 70 patients (6%) and AEs in 70 patients (6%). These AEs comprised clinical AEs in 29 patients (2.6%) (general malaise n=11, skin reactions n=11, arthralgia n=4, other n=3) and laboratory toxicity in 41 patients (3.5%).

Detection rate of myelotoxicity and hepatotoxicity

Overall, toxicity was detected in 2.030 (16%) of 12.391 laboratory assessments. During follow-up, 546 patients (48%) had signs of toxicity in one or more laboratory assessments. No difference was observed in monitoring rates between patients with or without laboratory toxicity (p=0.259). Myelotoxicity was observed in 370 patients (33%) in 1066 assessments. Overall detection rate of myelotoxicity was 8.6% i.e. 7.6% for mild myelotoxicity, 0.9% for moderate myelotoxicity and 0.04% for severe myelotoxicity (**Table 2**). Hepatotoxicity was present in 275 patients (24%) in 950 assessments with an overall detection rate of 7.7% i.e. 6.7% for mild hepatotoxicity, 0.8% for moderate hepatotoxicity and 0.1% for severe hepatotoxicity (**Table 2**).

Table 1. Baseline Characteristics

	N=1132
Male sex, n (%)	500 (44)
Age (yr), median [IQR]	37 [26-49]
Age at IBD diagnosis (yr), median [IQR]	26 [20-36]
Weight (kg), median [IQR][a]	73 [64-85]
Diagnosis, n (%) Ulcerative colitis Crohn's disease IBD-unclassified	363 (32) 736 (65) 33 (3)
CD – Montreal age at diagnosis, n (%) A1 <17 years A2 17-40 years A3 >40 years	93 (13) 508 (69) 135 (18)
CD – Montreal localization, n (%) L1 Ileal L2 Colonic L3 Ileocolonic L4 Upper GI disease Perianal involvement	239 (32) 162 (22) 335 (46) 47 (6) 192 (26)

Table 1. Continued.

	N=1132
CD – Montreal disease behavior B1 Non-stricturing, non-penetrating B2 Stricturing B3 Penetrating	383 (52) 212 (29) 141 (19)
UC – Montreal disease extent[a,b] E1 Proctitis E2 Left-sided E3 Extensive colitis	27 (7) 123 (33) 228 (60)
History of IBD related surgery, n (%)	334 (30)
Current smokers, n (%)[a]	241 (21)
Thiopurine therapy AZA MP	843 (74) 289 (26)
Thiopurine dose (mg/kg), median [IQR][a,c] AZA, adjusted[,d] MP	0.9 [0.8-1.0] 0.8 [0.6-1.0]
Concomitant IBD medication, n (%) 5-ASA Allopurinol+AZA / Allopurinol+MP	481 (42) 382 (34) 57 (5) / 42 (4)
TPMT genotype, n (%) Normal Heterozygote mutation Homozygote mutation Unknown	163 (14.4) 29 (2.6) 1 (0.1) 939 (82.9)

IBD, inflammatory bowel disease; IQR, interquartile range; kg, kilogram; AZA, azathioprine; MP, mercaptopurine; mg, milligram; 5-ASA, 5-Aminosalicylic acid; TPMT, thiopurine S-methyltransferase. a limited data for weight, n=1124; for Montreal disease extent, n=369; for smoking status n=1020; for thiopurine dose, n= 1124; for TPMT genotype, n=193; b Combined for UC /IBDU. c Excluding patients with concomitant use of allopurinol (n=99). d Adjusted AZA drug dose represents the equivalent pharmaceutical MP dose with a conversion factor of 2.08.

Table 2. Detection rate of laboratory toxicity

	Patients N=1 132	**Overall assessments** N=12 874
Median monitoring frequency / treatment year, n (IQR)	3.1 [2.2-3.9]	-
Detection of laboratory toxicity, n (%)	546 (48.2)	2030 (16.4)
Myelotoxicity, n (%) Mild Moderate Severe	370 (32.7) 284 (25.1) 82 (7.2) 4 (0.4)	1066 (8.6) 945 (7.6) 116 (0.9) 5 (0.04)
Hepatotoxicity, n (%) Mild Moderate Severe	275 (24.3) 229 (20.2) 38 (3.4) 8 (0.7)	950 (7.7) 836 (6.7) 101 (0.8) 13 (0.1)

IQR, interquartile range;

Incidence rate of myelotoxicity and hepatotoxicity

Myelotoxicity

The overall incidence rate of myelotoxicity was 7.1% per treatment year, specifically 5.2% for mild myelotoxicity, 1.8 % for moderate myelotoxicity and 0.1% for severe myelotoxicity (**Figure 1A**). In addition to 9% of patients (n=129) that showed myelotoxicity at baseline, cumulative incidence rates of myelotoxicity on maintenance thiopurine therapy were 12% at 1 year, 24% at 3 years, and 29% at 5 years of follow up. Median time to the development of myelotoxicity was 6 months from baseline [IQR 0-20]. The majority of detected myelotoxicity in laboratory assessments was classified as mild toxicity (945/1066 assessments, 89%) and comprised leukopenia in 97% (1032/1066 assessments).

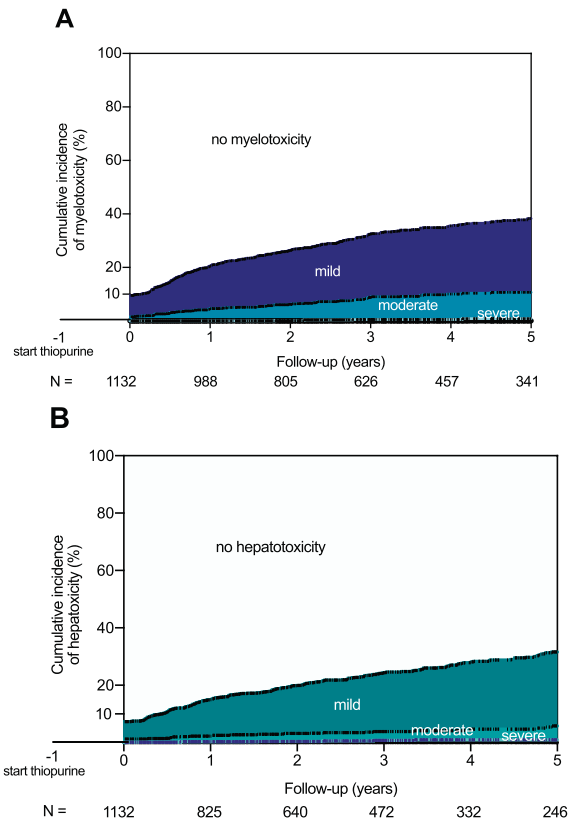

Figure 1. Incidence rate of laboratory toxicity. Cumulative incidence of laboratory toxicity, stratified according to severity of myelotoxicity (A) or hepatotoxicity (B). A) Patients were categorized based on the most severe value of myelotoxicity detected during follow up, with time to event set at first event in the corresponding category of severity. The dark blue area represents severe myelotoxicity (n=6); the blue area represents moderate myelotoxicity (n=83); the light blue area represents mild myelotoxicity (n=282); the light grey area represents patients without myelotoxicity (n=761). B) Patients were categorized based on the most severe value of hepatotoxicity detected during follow up, with time to event set at first event in the corresponding category of severity. The dark green area represents severe hepatotoxicity (n=8); the green area represents moderate hepatotoxicity (n=38); the light green area represents mild hepatotoxicity (n=253); the light grey area represents patients without hepatotoxicity (n=833).

Concomitant allopurinol treatment was a risk factor for myelotoxicity when compared to patients without laboratory toxicity (HR 1.59, 95% CI 1.04-2.43, P<0.034) (**Table 3**). A borderline significant interaction was observed between MP and allopurinol (p=0.08)

Hepatotoxicity

The overall incidence rate of hepatotoxicity was 5.1% per treatment year i.e. 3.8% for mild hepatotoxicity, 1.1 % for moderate hepatotoxicity and 0.2% for severe hepatotoxicity (**Figure 1B**). In addition to the 8% of patients (n=91) that showed hepatotoxicity at baseline, cumulative incidence rates of patients that showed signs of hepatotoxicity were 6% at 1 year, 14% at 3 years, and 21% at 5 years of follow up. Median onset of hepatotoxicity was 9 months from baseline [IQR 0-26]. In univariate and multivariate Cox regression analyses treatment with MP (HR 1.40 95% CI 1.10-1.78, P<0.006) and concomitant use of allopurinol (HR 2.73 95% CI 1.56-4.79, P<0.0001) were associated with an increased risk of hepatotoxicity in long-term thiopurine treatment (**Table 3**). No interaction was observed between MP and allopurinol.

In univariate and multivariate Cox regression analyses, male gender (hazard ratio (HR) 1.302; 95% confidence interval (CI) 1.10-1.54, P<0.009), treatment with MP (HR 1.56 95% CI 1.30-1.87, P<0.0001) and diagnosis of UC/IBDU (HR 1.27 95% CI 1.09-1.54, P<0.003) were associated with an increased risk of laboratory toxicity of any type in long-term thiopurine treatment (**Table 3**). No differences in overall toxicity, myelotoxicity and hepatotoxicity were observed in patients with TMPT abnormalities. Notably, thiopurine dose in patients with intermediate TPMT activity (0.78mg/kg +/- 0.27) was significantly lower than in patients with normal TPMT activity (0.90 mg/kg +/- 0.26) (P=0.027).

Clinical consequences of laboratory toxicity

Therapy adjustments for toxicity

After detection of laboratory toxicity, therapy adjustments were performed in 83 patients (7.3%) after a median follow-up time of 1.8 years [IQR 0.5-3.5]. Overall, 0.7% (83/12.391) of laboratory assessments in this cohort resulted in a therapy adjustment. These therapy adjustments comprised therapy cessation (n=28, 34%), dose reductions (n=46, 55%) and switch to LDTA therapy (n=9, 11%) (**Table 4**). Reasons for therapy cessation in these 28 patients were myelotoxicity (n=14), hepatotoxicity (n=9) and combined toxicity (n=5). Dose reductions were performed for myelotoxicity in 38/46 patients (83%), hepatotoxicity in 7/46 (15%) patients and combined toxicity in 1/46 patients (2%). Treatment with LDTA was initiated for hepatotoxicity in 6/9 (67%) patients and for myelotoxicity in 3/9 patients (33%). The overall incidence rate for treatment adjustment in patients on maintenance thiopurine treatment after detected laboratory toxicity was 1.9% per treatment year.

Table 3. Uni- and multivariate Cox-proportional hazards regression analysis to explore factors associated with development of laboratory toxicity (A) myelotoxicity (B) or hepatotoxicity (C) in patients on long term maintenance thiopurine therapy.

	Laboratory toxicity				Myelotoxicity		Hepatotoxicity			
	Univariate analysis		Multivariate analysis		Univariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Male gender	1.30 (1.10-1.54)	0.002*	1.29 (1.09-1.52)	0.009	1.13 (0.92-1.40)	0.240	1.13 (0.90-1.42)	0.309		
Mercaptopurine[a]	1.56 (1.30-1.87)	<0.0001*	1.56 (1.30-1.87)	<0.0001	1.02 (0.81-1.29)	0.869	1.46 (1.15-1.85)	0.002*	1.40 (1.10-1.78)	0.006
Ulcerative colitis/ IBDU[b]	1.30 (1.09-1.54)	0.003*	1.25 (1.06-1.49)	0.003	1.10 (0.89-1.35)	0.399	1.07 (0.84-1.35)	0.601		
Previous surgery	0.90 (0.75-1.09)	0.821			0.96 (0.75-1.19)	0.626	0.89 (0.70-1.15)	0.377		
Current smoking	0.80 (0.64-1.01)	0.055			0.80 (0.60-1.07)	0.795	1.12 (0.83-1.51)	0.460		
Concomitant allopurinol	1.45 (0.93-2.27)	0.317			1.59 (1.04-2.43)	0.034	1.75 (1.16-2.66)	0.008*	2.73 (1.56-4.79)	<0.0001
Concomitant 5-ASA	1.12 (0.90-1.39)	0.102			1.04 (0.82-1.33)	0.751	0.83 (0.62-1.10)	0.186		

HR, hazard ratio; CI, confidence interval; IBDU, Inflammatory bowel disease unclassified; 5-ASA, 5-aminosalicylic acid. Laboratory toxicity was defined as any type of myelotoxicity and/or hepatotoxicity.

* Covariates with a p-value <0.2 were included in the multivariable regression analysis

[a]Drug type, mercaptopurine vs. azathioprine [b]IBD diagnosis, UC/IBDU vs. Crohn's disease.

Table 4. Clinical consequences of laboratory toxicity

	Patients N= 1 132	**Laboratory assessments** N=12 391
Therapy adjustments, n (%)	83 (7.3)	83 (0.7)
Cessation	28 (2.5)	28 (0.2)
Dose reduction [b]	46 (4.1)	46 (0.4)
LDTA therapy	9 (0.8)	9 (0.1)
Median time to adjustment, IQR	1.8 [0.5-3.5]	-
Indication therapy adjustment, n (%)	55 (3.0)	55 (0.4)
Myelotoxicity	17 / 36 / 2	
mild / moderate / severe	22 (3.9)	22 (0.2)
Hepatotoxicity	14 / 6 / 2	
mild / moderate / severe	6 (0.4)	6 (0.05)
Myelotoxicity and hepatotoxicity	2 / 3 / 1	
mild / moderate / severe		
Diagnostic procedures, n (%)	111 (9.8)	154 (1.2)
Laboratory assessment[a]	86 (7.6)	121 (1.0)
Ultrasound[a]	19 (1.8)	22 (0.2)
MRI/MRCP	6 (0.5)	6 (0.05)
Ultrasound and Liver biopsy[b]	5 (0.4)	5 (0.04)
Indication diagnostic procedure, n (%)	56 (4.9)	67 (0.5)
Myelotoxicity	47 (4.2)	77 (0.6)
Hepatotoxicity	8 (0.7)	10 (0.1)
Myelotoxicity and hepatotoxicity		
Complications, n (%)	12 (1.1)	12 (0.1)
Infections	7 (0.6)	7 (0.06)
Blood and lymphatic system	4 (0.4)	4 (0.03)
Gastro-intestinal disorders	1 (0.1)	1 (0.01)

IQR, interquartile range; MRI, magnetic resonance imaging; MRCP, Magnetic Resonance Cholangio-Pancreatography.
Complications included 6 grade 1 complications, 9 grade 2 complications and 11 grade 3 complications.
a13 patients received extra laboratory assessments and ultrasound. bperformed within a trial.

Cumulative incidence rate of therapy adjustments were 2.5% at 1 year, 6.2% at 3 years, 8.9% at 5 years, and 15.4% at 10 years of follow up (**Figure 2**). A higher thiopurine dose (HR 3.1 95% CI 1.5-6.4, P<0.004), higher annual monitoring frequency (HR 1.01 95% CI 1.00-1.01, P<0.0001) and higher number of aberrant assessments (HR 1.04 95% CI 1.04-1.05, P<0.0001) were independently associated with therapy adjustments. No correlation was observed between annual monitoring rate and the number of aberrant assessments (Spearman correlation R=0.09, p=0.762).

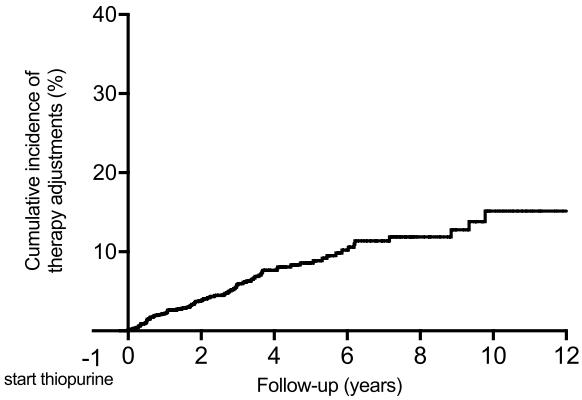

Figure 2. Cumulative incidence of therapy adjustments during thiopurine treatment for laboratory toxicity.

Monitoring rate

The median monitoring frequency was 3.1 laboratory assessments per treatment year (IQR 2.2-3.9) with a slight decreasing trend in monitoring rate over time, ranging from 3.1 assessments in the first year of follow-up (monitoring interval 3.9 months) to 2.0 assessments per year (monitoring interval 6.0 months) after 6 years of follow-up. The mean monitoring interval in patients receiving a therapy adjustment (3.3 months, SD 1.8) was shorter (i.e. more stringent) than in patients without an adjustment (4.1 months, SD 2.1, p<0.0001) . Also, the mean monitoring interval in patients receiving a therapy adjustment was shorter than in patients with laboratory toxicity but without an adjustment (4.2 months, SD 2.0, p<0.0001). No difference was observed in the mean monitoring interval in patients with laboratory toxicity (4.0, SD 2.0) and patients without toxicity (4.1, SD 2.2) (P=0.757). In patients receiving a therapy adjustment, the antecedent-monitoring interval was not significantly different from the mean monitoring interval (3.8 months (SD 3.7) vs. 3.3 months (SD 1.8), p=0.154). When comparing incidence rate of toxicity in patients on the most stringent monitoring regime (upper quartile, mean monitoring interval 2.3 months) with patients on the most liberal monitoring regime (lower quartile, mean monitoring interval 6.3 months)) no differences were observed in overall laboratory toxicity. However, the incidence rates of moderate leukopenia and severe hepatotoxicity were higher in patients on a stringent monitoring regimen than in patients on a liberal monitoring regimen (12% vs. 4.2%, p=0.001) (5% vs 0%, p=0.025). Details are depicted in **Table 5**. Cumulative incidence rate of therapy adjustments throughout follow up was higher in patients on a stringent monitoring regimen than on a liberal monitoring regimen (**Supplementary Figure 1**).

Table 5. Detection rate of laboratory toxicity stratified for monitoring rate

	Patients N=1 132	**Liberal monitoring** N=283	**Stringent monitoring** N=283	**P-value**
Median monitoring rate/treatment yr, n (IQR)	3.1 [2.2-3.9]	1.8 [0.0-2.0]	4.8 [4.3 – 6.3]	
Mean monitoring interval (months), (SD)	4.1 (2.1)	6.3 (2.5)	2.3 (1.3)	<0.0001
Detection of laboratory toxicity, n (%)	546 (48.2)	117 (41.3)	136 (48.0)	0.128
Myelotoxicity, n (%)	370 (32.7)	75 (26.5)	100 (35.3)	0.023
Mild	284 (25.1)	63 (22.3)	65 (23.0)	0.841
Moderate	82 (7.2)	12 (4.2)	34 (12.0)	0.001
Severe	4 (0.4)	0 (0.0)	1 (0.4)	0.317
Hepatotoxicity, n (%)	275 (24.3)	51 (18.0)	69 (24.4)	0.081
Mild	229 (20.2)	45 (15.9)	53 (18.7)	0.347
Moderate	38 (3.4)	7 (2,5)	11 (3.9)	0.338
Severe	8 (0.7)	0 (0.0)	5 (1.8)	0.025
Treatment related complications	12 (1.1)	1 (0.4)	8 (2.8)	0.019

IQR, interquartile range; SD, standard deviation. Liberal monitoring group comprises the lower quartile (lowest monitoring rate/treatment year) of the study population. Stringent monitoring group comprises the upper quartile (highest monitoring rate/treatment year) of the study population. P-values concern differences between liberal and stringent monitoring. Treatment-related complications comprised complications associated with concurrent laboratory toxicity.

Diagnostic procedures

Additional diagnostic procedures following established laboratory toxicity were performed in 154 aberrant laboratory assessments (7.6%) in 111 patients (9.8%). Overall, 1.2% of all assessments resulted in additional diagnostic procedures (**Table 4**). Most physicians followed up on detected toxicity through extra laboratory assessments (121 assessments in 86 patients), and 55% of these additional assessments were triggered by myelotoxicity. Ultrasound was performed after established hepatotoxicity in 27 patients (2.4%). No cases of nodular regenerative hyperplasia were reported.

Complications

Clinical treatment-related complications with concurrent laboratory toxicity were detected in 12 patients (1.1%) in this cohort. The incidence rate of treatment-related complications with concurrent laboratory toxicity was 0.27% per treatment year (**Table 4**). Details on treatment-related complications are depicted in **Table 6**.

Table 6. Clinical treatment-related complications with concurrent laboratory toxicity

	Total cohort N=1 132	**Myelotoxicity** N=14	**Hepatotoxicity** N=6	**Myelotoxicity + Hepatotoxicity** N=1
Cytomegalovirus infection n (%)	6 (0.5)	0 (0.0)	5 (0.4)	1 (0.1)
Blood transfusion[a] n (%)	2 (0.2)	2 (0.2)	0 (0.0)	0 (0.0)
Hospital admission for gastroenteritis n (%)	2 (0.2)	2 (0.2)	0 (0.0)	0 (0.0)
Jaundice[b] n (%)	1 (0.1)	0 (0.0)	1 (0.1)	0 (0.0)

[a] Patients received a transfusion with packed red blood cells or iron for pancytopenia.
[b] Patient at stable thiopurine dose presented with jaundice and hepatotoxicity after weight loss due to a sleeve gastrectomy.

Complications were more often observed in patients treated with MP than in AZA-treated patients (n=6, 2.1% versus n=6, 0.7%, P=0.015). Complication rate was higher in patients on a stringent monitoring regimen than in patients on a liberal monitoring regimen (2.8% vs 0.4%, P=0.019) (**Table 5**). Stringent monitoring remained associated with a higher complication rate when excluding all laboratory assessments after the onset of complications. Strikingly, 3/12 patients (25%) developed mild myelotoxicity, 1/12 (13%) patients developed moderate myelotoxicity prior to complications and 8/12 patients (67%) presented with clinical symptoms and had no signs of toxicity in preceding laboratory assessments. Five of these 8 patients received stringent laboratory monitoring. No mortality was observed.

DISCUSSION

Frequent laboratory monitoring is advised throughout thiopurine maintenance treatment to detect myelotoxicity and hepatotoxicity. However, laboratory toxicity usually develops within the first few months of treatment and the reported incidence rate in maintenance treatment is low. This large cohort study has demonstrated that current laboratory monitoring regimen has limited value in patients on thiopurine maintenance monotherapy for at least 1 year. Although the incidence of myelotoxicity was 7.1% and of hepatotoxicity 5.2% per treatment year, laboratory assessment had little impact on clinical decision making. Only 0.7% of laboratory assessments resulted in therapy adjustments, and only 1.4% to further diagnostic procedures. Furthermore, severe treatment-related complications, such as infection and hospitalisation, attributed to concurrent laboratory toxicity are rare and these complications were not prevented with current monitoring regimen as the majority of cases were not preceded by laboratory toxicity in previous assessments.

The incidence of myelotoxicity and hepatotoxicity is line with previous studies, although there is no universal standard definition of laboratory toxicity, and small sample size and short

follow up in previous studies for comparison.[20-22] Myelotoxicity incidence in our study of 7.1% per treatment year of follow up is based on stringent criteria and ranged from 5.1% for mild myelotoxicity, 1.8 % for moderate myelotoxicity to 0.1% for severe myelotoxicity. Myelotoxicity incidence was evaluated in a review of studies in which myelotoxicity definition varied from leukocyte count of <2.0 x 10e9/l to <4.5 x 10e9/L. As expected, a higher incidence rate of 11% in the first year of treatment was reported than in our study. Nevertheless, the overall range of myelotoxicity incidence of 3 to 8% per treatment year is compatible with our findings for maintenance treatment.9 In our study, hepatotoxicity incidence was 5.1% per treatment year. While incidence rates of 0 to 17% have been reported in other studies, possibly attributable to the heterogeneity of definitions, our findings are in line with large cohort studies that have reported incidence rates of 4 and 7%.8,20,23,24 Furthermore, the moderate to severe hepatotoxicity rate of 1.3% per treatment year in our cohort is in line with the rate of 1% per treatment year in other studies.[8,10]

In this study, the most stringent monitoring regimen was associated with increased incidence rates in moderate myelotoxicity and severe hepatotoxicity compared to the most liberal monitoring regimen. These results should be interpreted with caution, as the causal relation is unclear. The probability of detecting toxicity will increase with more frequent monitoring. Yet, abnormal laboratory values will urge physicians to increase the monitoring frequency to follow up on toxicity. Thus, it cannot be concluded that more frequent monitoring is more likely to pick up toxicity, or that previously established toxicity increases the monitoring frequency without direct clinical consequences.

In our study, fewer therapy adjustments were made to thiopurine treatment as a result of laboratory toxicity than were reported in other studies.[25-28] Our study focused on the consequences of laboratory monitoring after 1 year of maintenance therapy in routine clinical practice and not the consequences of laboratory findings shortly after initiation of thiopurines. In our study, laboratory toxicity resulted in therapy adjustment in 7% of patients during follow up compared to reported rates of up to 15% in other studies. Furthermore, only 2.8% of patients in our cohort had to be withdrawn from therapy because of laboratory toxicity compared to 6 to 13% of patients in other studies.[25-28] In line with the high risk of laboratory toxicity after start of thiopurines compared to maintenance treatment, these results indicate that therapy withdrawal due to laboratory toxicity usually occurs within the first months of treatment rather than during maintenance treatment.[8,12,23]

Our study has shown that mild toxicity is often disregarded in clinical practice with respect to additional diagnostic procedures. Laboratory results may have been disregarded because of a presumed low association between mild myelotoxicity and increased risk of infections, and possibly favourable outcome of mild leukopenia on therapeutic effectiveness of thiopurines.[29]

A low risk of laboratory toxicity-associated complications was observed. Similarly, mild hepatotoxicity was often disregarded in our cohort probably because the association of transient hepatotoxicity with chronic liver disease is questionable. Laboratory toxicity-associated complications were detected in 1% of patients in our cohort. Only 33% of patients had signs of laboratory toxicity in previous assessments. We observed that 67% of these patients received stringent laboratory monitoring (upper quartile (highest) annual monitoring rate of the study population). Treatment-related complications were more often observed in patients who received stringent monitoring than in those who received more liberal monitoring (lower quartile annual monitoring rate). As such, stringent monitoring does not prevent myelotoxicity-related or hepatotoxicity-related complications. These observations are of considerable significance as the clinical impact of detecting laboratory toxicity in maintenance therapy is low, and thus routine monitoring is of limited benefit.

Mercaptopurine was found to be associated with a higher risk of myelotoxicity than AZA.[23,30] This finding may be attributed to higher dosing of MP than AZA in IBD patients, because of little variation in pharmaceutical dosages in MP tablets and relatively higher recommended dose in official clinical guidelines than for AZA when correcting for bioavailability.[30] This association was not confirmed in maintenance treatment in our cohort, presumably because of low dosing of thiopurines in general and MP in particular (lower dosing than recommended in guidelines). In line with previous studies, MP was associated with hepatotoxicity (HR 1.40) in our cohort. This may well be influenced by the percentage of MP users (15%) receiving concomitant therapy with allopurinol. Patients treated with LDTA showed higher detection rates of hepatotoxicity in multivariate analysis but this interaction was not confirmed by statistical analysis. However, the likelihood of detecting hepatotoxicity in patients on LDTA is higher because some patients possibly started on allopurinol shortly before inclusion in the study and hepatotoxicity had not (yet) normalised in the course of combination therapy.

To our knowledge, our study is the first to describe the consequences of laboratory monitoring throughout thiopurine maintenance monotherapy in a large cohort. Limitations relate mostly to the retrospective nature of this study. Firstly, we may have underestimated the incidence of laboratory toxicity because prospectively identified time points were not evaluated. True myelotoxicity can only be identified when a full blood count is completed. In addition, the absolute neutrophil count seems to be an important hematologic value in assessing susceptibility to infections.[31] As neutrophils were not routinely measured, transient episodes of myelotoxicity may not have been recorded. On the other hand, all detected laboratory toxicity was attributed to thiopurine therapy but other medication or viral infections are also associated with myelotoxicity.[32,33] Also, an increased risk of hepatotoxicity has been reported in IBD associated with other causal factors than thiopurine use, such as fatty liver disease.[24] Patients with a known history of liver disease were excluded in the analysis, but undiagnosed fatty liver disease in the patient population cannot be ruled out. High levels of the active thiopurine metabolite 6-thioguanine nucleotides are associated with myelotoxicity, and the byproducts 6-methyl mercaptopurine ribonucleotides are associated with both myelotoxicity

and hepatotoxicity.[34,35] Considerations of thiopurine metabolite levels and adverse events are hampered because thiopurine metabolites were not routinely measured and thus not included in the analysis. Secondly, data from 6 hospitals were included and both cessation and dose reductions were left to the discretion of the treating physician. Thus, the monitoring regimen and the clinical consequences after toxicity detection reflect the daily practice of these physicians. A clinician's motivation to perform therapy adjustments or diagnostic procedures could be influenced by experience or patient-related factors. It is likely that repeated detection of laboratory abnormalities led the treating physician to adjust treatment. In addition, both patients and physicians influenced compliance to laboratory monitoring regimen. Thirdly, evaluation of clinical consequences of laboratory toxicity was hampered by the inability to discriminate between routine laboratory assessment for monitoring thiopurine therapy and laboratory assessments requested by other clinicians. This might also have led to overestimation of the monitoring frequency. Finally, almost 75% of patients were followed in a referral centre, and thus our population probably includes patients with a more complicated disease course or more comorbidity with a possible increased risk of laboratory toxicity. Our results apply to patients on thiopurine monotherapy. The risk of laboratory toxicity in patients on combination therapy with biologic agents was not investigated, and the risk in this population may be higher.[36]

As thiopurine-induced laboratory toxicity occurs more frequently in the first months of therapy, strict laboratory monitoring of the blood count and LTs in the first year of treatment as recommended in current guidelines seems justified.[9,10] Regular laboratory monitoring is recommended at 2 to -3 month intervals during maintenance thiopurine treatment.[16,22] These recommendations are largely based on concern about possible complications following late-onset toxicity, especially leukopenia. However, the cost-effectiveness of this schedule is not evidence-based.[15,16] Laboratory monitoring practices in this large real-life cohort were more liberal than recommended in the ECCO guideline; at 4-month intervals (our study) versus at 2-3 month intervals (ECCO guideline). The results of our study demonstrate that after 1 year of thiopurine treatment, monitoring at 4-month intervals rarely leads to therapy adjustments and more importantly is rarely associated with treatment-related complications. Also, (frequent) monitoring after 1 year of treatment does not seem to prevent laboratory toxicity-related complications, as preceding laboratory assessments were unremarkable in 67% of cases. Therefore, the firm conclusion can be drawn that the recommended monitoring frequency may be reduced to an interval of less than 4 months. We speculate that a monitoring regimen at 6-month intervals is sufficient in patients after 1 year of thiopurine treatment. This assumption is supported by the small number of complications in patients on the least frequent monitoring regimen (i.e. lower quartile based on annual in patients monitoring frequency). Also, we hypothesize that reducing laboratory monitoring to 6-month intervals could decrease patient burden and healthcare costs (Supplementary data 1). In order to confirm that laboratory monitoring at 6-month intervals is non-inferior to 4-month intervals, prospective evaluation in an impractically large study population with several years of follow-up would be required. Accurate risk stratification for complications, based on detected laboratory toxicity, is

hampered by the heterogeneity of data in this study. It is unlikely that this will be avoided by a prospective design, as the detection of laboratory toxicity is expected to influence both treatment and monitoring. Therefore, a prospective cohort study will probably not provide the required data to test this hypothesis.

In conclusion, this study has demonstrated a limited yield of current laboratory monitoring practices in maintenance thiopurine monotherapy in IBD patients. Firstly, laboratory monitoring in clinical practice was less frequent than advised in current guidelines. Secondly, severe myelotoxicity and hepatotoxicity are uncommonly detected. Thirdly, this study showed that treating physicians tend to disregard aberrant laboratory findings, and were not inclined to adjust therapy or perform additional diagnostic evaluation. Finally, complications associated with laboratory toxicity occurred rarely, and most complications developed unpredictably and could not be avoided by frequent monitoring. Reducing laboratory-monitoring in thiopurine maintenance therapy after 1 year of treatment to less than a 4-month interval seems sufficient and could result in reduced patient burden and health-care costs.

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SUPPLEMENTARY DATA

Supplementary data 1. Hypothesis of cost-effectiveness analysis
Patients: Number of patients in the Netherlands receiving long-term maintenance thiopurine therapy (>1 year) estimated at 18.500.

Direct health-care costs (i.e. costs for monitoring-related health care resource utilization such as laboratory assessments, analysis, evaluation by physician)
€70 per laboratory assessment 37-39

Indirect health-care costs (i.e. travel costs, productivity loss)
€77.50 per laboratory assessment 37-39

Costs associated with thiopurine-induced complications
Direct costs: €3000 per patient per event
Indirect costs: €1500 per patient per event
€4500 per patient per event 39-41

Hypothesis: routine laboratory monitoring at 3-month intervals will prevent 1% more events (complication) than monitoring a 6-month intervals.

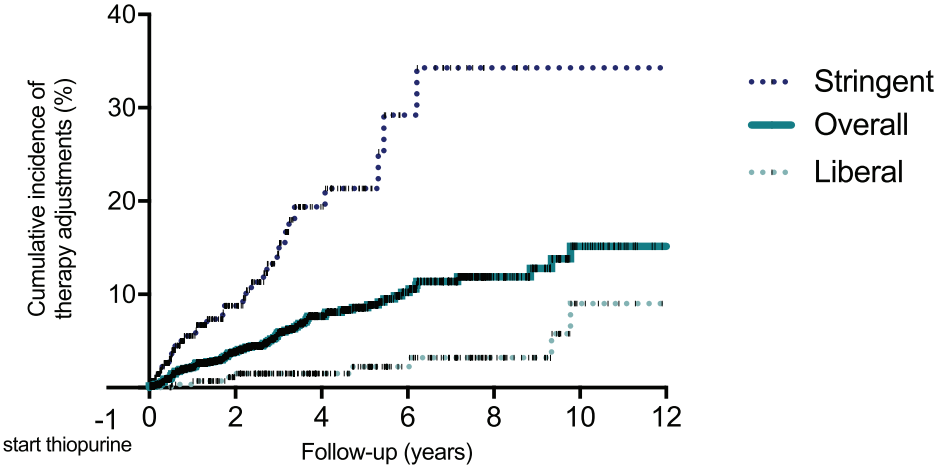
Intervention (monitoring at 6-month intervals)
Direct costs:
monitoring(€70*2) + complication costs (1.3% * 18.500 patients * €4500) = €2.590.000 + €1.082.250 = €3.672.250/yr= €199/patient/year.

Indirect costs:
productivity loss: (2*2hours= 2 x €25,50) + travel costs (2*€26.5) = €155/patient/year.

Control (monitoring at 3-month intervals)
Direct costs:
monitoring(€70*4) + complication costs (0.3% * 18.500*€4500)= €5.180.000 + €249.750 = € 5.429.750/yr = €294/patient/year.

Indirect costs:
productivity loss: (4*2hours= 2 x €25,50) + travel costs(4*€26.5) =€ 310/patient/year.

Anticipated point estimate for the difference in mean cost of intervention versus control:
(294+310) – (199+155) = € 250 per patient per treatment year.



Supplementary Figure 1. Cumulative incidence of therapy adjustments during thiopurine treatment for laboratory toxicity stratified for monitoring frequency. The blue line represents the cumulative incidence of therapy adjustments during thiopurine treatment in the entire cohort; i.e. overall (n=1132). The dotted dark blue line respresents the cumulative incidence of therapy adjustments during thiopurine treatment in the highest quartile monitoring frequency; i.e. stringent (n=283). The dotted light blue line respresents the cumulative incidence of therapy adjustments during thiopurine treatment in the lowest quartile monitoring frequency; i.e. liberal (n=283).

CHAPTER 6

SEX IS ASSOCIATED WITH ADALIMUMAB SIDE-EFFECTS AND DRUG SURVIVAL IN CROHN'S DISEASE PATIENTS

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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 treatment success (ongoing ADA use). Reasons for stopping ADA were recorded. Kaplan-Meier analysis and Cox regression were used to assess treatment success.

Results: The cohort consisted of 107 female and 81 male patients. Median follow-up was 6.0 years (range 0.3-9.2). Treatment success 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 treatment success. 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 treatment success 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-a).1,2 Adalimumab (ADA) is a recombinant human IgG1 monoclonal antibody against TNF-a 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 psoriasis12 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.

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 previously14. 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 $\chi 2$ 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 Center Rotterdam, the Netherlands.

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 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%, $\chi 2$ 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).

Table 1. Patient characteristics at baseline

	Complete cohort (N=188)	**Males** (N=81)	**Females** (N=107)	**P-value†**
Median age at diagnosis (years, range)	24 (5-74)	24 (10-74)	23 (5-63)	0.931
Median age at start of ADA (years, range)	36 (18-81)	36 (18-81)	36 (18-66)	0.201
Median disease duration at start of ADA (years, range)	9 (0-49)	10 (1-44)	8 (0-49)	0.532
Montreal localization, n (%) L1 L2 L3	44 (23.4) 53 (28.2) 91 (48.4)	22 (27.2) 30 (28.40) 36 (44.4)	22 (20.6) 30 (28.0) 55 (51.4)	0.290 0.957 0.345
Montreal disease behavior, n (%) B1 B2 B3	104 (55.3) 46 (24.5) 38 (20.2)	43 (53.1) 20 (24.7) 18 (22.2)	61 (57.0) 26 (24.3) 20 (18.7)	0.592 0.951 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 start of ADA, n (%) None, 5-ASA and/or steroids 5-ASA, steroids and immunosuppressives 5-ASA, steroids, immunosuppressives and biologicals	15 (8.0) 71 (37.8) 102 (54.3)	1 (1.2) 37 (45.7) 43 (53.1)	14 (13.1) 34 (31.8) 59 (55.1)	**0.003** **0.052** 0.780
Concomitant medication at start of ADA, n (%) Corticosteroids Immunosuppressives*	84 (44.7) 74 (39.4)	39 (48.1) 36 (44.4)	45 (42.1) 38 (35.5)	0.405 0.215
Previous intestinal surgery, n (%)	73 (38.8)	31 (38.3)	42 (39.3)	0.891
BMI kg/m2, 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**

ADA, adalimumab; 5-ASA, 5-Aminosalicylic acid; 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.

Reasons for starting ADA therapy (**Table 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).

Table 2. Reasons for initiating adalimumab therapy

	Total cohort (n=188)	**Males** (n =81)	**Females** (n=107)	**P-value‡**
Reason for initiating adalimumab therapy				
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.

Clinical outcomes to ADA therapy

Figure 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%, $\chi 2$ 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. 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%, $\chi 2$ 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%, $\chi 2$ P=0.016).

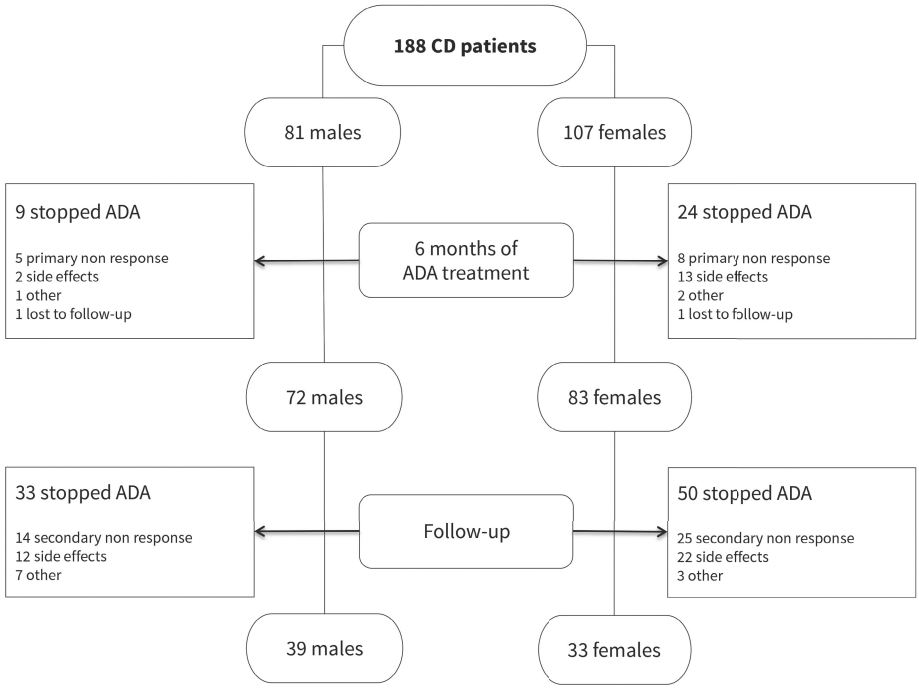

Figure 1. Flowchart depicting outcomes in the cohort, divided by male and female patients.

Similarly, in Kaplan-Meier analysis a difference was seen in male and female ADA continuation rates (**Figure 2**, log-rank P=0.006). Additionally, in univariable and multivariable Cox proportional hazard models (**Table 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.

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%, $\chi 2$ 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 greatersuccess percentage in male than female patients (83.7% vs 60.7%, $\chi 2$ P=0.011).

Table 3. Univariable and multivariable Cox proportional hazard regression model.

	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).

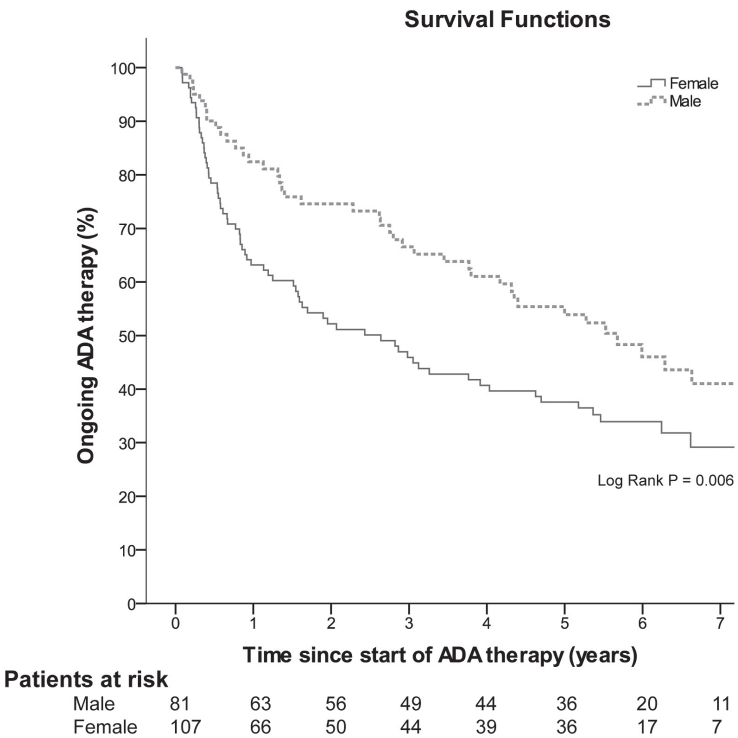

Figure 2. Kaplan-Meier survival curve, displaying ongoing adalimumab treatment in males and females, over 7 years. The difference between ongoing treatment in males and females is significant (log-rank P=0.006).

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 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, $\chi 2$ 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%, $\chi 2$ 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 (n=69), were present prior to ADA therapy (n=42), or were clearly not caused by ADA (n=12; 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 treatment11. 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 men20, probably due to sex related differences in sex hormone levels. However, CYP3A4 has been shown to be influenced by TNF-a21, 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 studies27,29, 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 treatment success. 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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SUPPLEMENTARY DATA

Supplementary Table 1. Adverse events reported during adalimumab use, ordered by frequency.

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

* e.g. cough, sore throat rhinitis, pneumonia; † e.g. conjunctivitis, episcleritis, blurred vision; ‡ Adverse events reported once (ordered alphabetically): alcohol intolerance, anemia, back pain, bloated feeling, constipation, dyspnea, ear infection, epilepsia, epistaxis, hypermenorrhea, hypertension, general malaise, migraine, peripheral edema, tendinopathy, thrombopenia, urticaria, vasculitis.

PART II

CERVICAL NEOPLASIA IN IBD

CHAPTER 7

INCREASED RISK OF HIGH-GRADE CERVICAL NEOPLASIA IN WOMEN WITH INFLAMMATORY BOWEL DISEASE: A CASE-CONTROLLED COHORT STUDY

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ABSTRACT

Background: Women with inflammatory bowel disease (IBD) may be at higher risk for cervical intraepithelial neoplasia (CIN). However, available data are conflicting and most studies lack longitudinal data. The aim of this study is to assess the risk of high-grade dysplasia and cancer (CIN2+) in IBD women and identify risk factors.

Methods: Clinical data from adult IBD women in a multicentre Dutch IBD prospective cohort (PSI) from 2007 onwards were linked to cervical cytology and histology records from the Dutch nationwide cytology and pathology database (PALGA) from 2000 to 2016. Patients were frequency matched 1:4 to a general population cohort. Standardized detection rates (SDR) were calculated for CIN and cancer. Longitudinal data were assessed to calculate CIN2+ risk during follow-up using incidence rate ratios (IRR), and persistent or recurrent CIN. IBD-specific risk factors for CIN2+ were identified in multivariable analysis.

Results: Cervical records were available from 2,098 IBD women (77%) and 8,379 in the matched cohort; median follow-up 13 years. CIN2+ detection rate was higher in the IBD cohort than in the matched cohort (SDR 1.27, 95%CI 1.05-1.52), especially in women aged 35-39 years (SDR 1.80, 95%CI 1.14-2.70). Excluding women with an abnormal screen at first available record, women with IBD had an increased risk of CIN2+ (IRR 1.66, 95%CI 1.21-2.25), and persistent or recurrent CIN during follow-up (OR 1.89, 95%CI 1.06-3.38). Risk factors for CIN2+ in IBD women were smoking and disease location (ileocolonic (L3) or upper-GI (L4)). CIN2+ risk was not associated with exposure to immunosuppressants.

Conclusion: Women with IBD are at increased risk for CIN2+ lesions. These results underline the importance of HPV vaccination and adherence to cervical cancer screening guidelines in IBD women, regardless of exposure to immunosuppressants.

INTRODUCTION

IBD is a chronic inflammatory disease characterized by an exaggerated and self-perpetuating immune response in the gut and extra-intestinal tissues. Over the past decades, immunomodulators and biological agents have become available widely for the treatment of Crohn's disease (CD) and ulcerative colitis (UC).[1,2] Due to their chronic inflammatory state and frequent use of immunosuppressive medication, patients with IBD are generally considered as immunocompromised.

Cervical cancer is the fourth most common type of cancer in women worldwide and virtually all cancers result from a persistent infection with high-risk types of the human papillomavirus (hrHPV). The development of cancer from a persistent hrHPV-infection follows a stepwise progression via two stages of squamous intraepithelial lesions (low and high SIL) equivalent to the histologic diagnosis of cervical intraepithelial neoplasia (CIN) 1 and CIN 2/3, respectively.[3,4,5] In immunocompromised women, impaired detection of oncogenic signals or decreased immunosurveillance might accelerate the progression of CIN to invasive cancer.[6] The risk of cervical neoplasia and cancer in women with IBD has been studied previously, however, results are conflicting. Some studies reported an increased incidence of cervical abnormalities,[7-11] whereas others did not find a significantly higher incidence amongst women with IBD.[12-15] These studies use different outcomes; solely cervical cytology results, or cervical dysplasia or cancer risk and both population-based and single center IBD cohorts were studied. In addition, most of these cohorts lack details on longitudinal follow up and detailed information on IBD disease characteristics. The current European Crohn's and colitis organization (ECCO) guideline recommends an intensified screening approach in immunocompromised IBD women[16] and American guidelines recommend intensified screening only in IBD women using immunosuppressive medication.[17, 18] However, these recommendations are based on low level of evidence.[18]

The aim of this study was to assess the detection rate and risk of CIN and cervical cancer in women with IBD as compared to the general Dutch female population and to assess the influence of IBD disease characteristics and exposure to immunosuppressive medication. A secondary aim of this study was to assess screening behavior and adherence to the cervical cancer screening program for women with IBD.

METHODS

Data collection

A nationwide cohort study was performed within the Dutch nationwide IBD biobank registry named Parelsnoer Institute (PSI). PSI started in 2007 as a collaborative project of the eight University Medical Centers in the Netherlands, and comprises clinical data that are collected with a standardized information model and biomaterial.[19] Clinical data from all female IBD patients in the PSI cohort were linked to data on cervical cytology and histology in the Dutch nationwide network and registry of histology and cytopathology (PALGA).[20] In PALGA, individuals are identified by a code comprised from birth date and the first eight letters of the surname. This code was used to link the PSI and PALGA databases. All cervical records between January 2000 and December 2016 were retrieved from the PALGA database, including indication for cytological assessment, i.e. within the national screening program or by other indications. Each woman with IBD from the PSI cohort was randomly frequency matched by age and year of first available cervical record in PALGA to four women from the general population. To correct for the higher prevalence of cervical lesions in women living in urbanized areas[21], the four-digit postal code from each woman was used to identify women living in low (<100,000 inhabitants) and high (>100,000 inhabitants) level urbanization areas. Matched women without cytological or histological result (i.e. hrHPV test only) within the study period were excluded.

Definitions and follow-up according to population cervical cancer screening

CIN and cervical cancer were coded according to the systemized nomenclature of medicine (SNOMED).[22] CIN1 was defined as mild dysplasia, CIN2 as moderate dysplasia, CIN3 as severe dysplasia or carcinoma in situ, and cervical cancer as invasive cervical squamous cell carcinoma or non-clear cell adenocarcinoma. CIN2+ was defined as the combination of CIN2, CIN3 and cervical cancer. Since only histological diagnoses were included as an endpoint in this study, the historical CIN classification was used instead of the two-tiered Bethesda classification for cytological screening.[23] The number of screening episodes in a 5-year period was calculated as a proxy of screening behavior. A screening episode started with a primary test and if abnormal or inconclusive, this primary test was followed by secondary test. An episode ended after 4 years following the primary test when no (adequate) follow-up test had been performed, or when follow-up had been completed according to the Dutch cervical cancer screening program.[24] Thus, by definition, post-diagnostic follow-up smears were attributed to the same episode as the diagnosed lesion. Screening behavior was measured for each woman by dividing the number of screening episodes by the number of 5-year follow-up periods (1: 0-5 years, 2: 5-10 years, 3: 10-15 years, 4: > 15 years) during follow-up.

Statistical analysis

Standardized detection ratios

The primary outcome was CIN2+ detection rate, defined as the percentage of episodes resulting in a histological diagnosis of CIN2+. Standardized detection ratios (SDRs) were

calculated by correcting the observed detection rates from the IBD cohort by the expected detection rates based on 5-year age categories, 5-year time periods and urbanization level. The expected detection rates were the calculated detection rates in the matched cohort. A two-tailed P-value <0.05 was considered statistically significant and 95% confidence intervals (CI) were calculated assuming a Poisson distribution.

Incidence rate ratios during follow-up

Follow-up for each woman started on the first available cervical record in the PALGA database (index date) and ended at 31st December 2016. Women were censored after the first occurrence of the highest grade of cervical neoplasia during follow-up or end of follow-up. Incidence rates (IR) per 100,000 person-years were calculated for both the IBD cohort and the matched cohort and incidence rate ratios (IRR) were computed. A sensitivity analysis was performed after exclusion of women with cervical neoplasia at the first screen within the study period. Kaplan Meier survival analyses were performed for the risk of CIN1 and CIN2+ diagnoses and statistical differences were calculated with a log-rank test. The effect of age on CIN2+ detection was visualised using attained age as time metric on the x-axis in a secondary analysis. Attained age was defined as the age at diagnosis of first occurrence of the highest CIN diagnosis during follow-up or age at end of follow-up. Cox proportional hazards regression analysis was performed to calculate hazard ratios (HRs) in order to quantify the effect of IBD on the risk of CIN2+ in the IBD cohort, adjusting for urbanization and screening behaviour.

Persistent or recurrent CIN lesions

Patients with persistent or recurrent CIN or CIN2+ lesions were identified by detection of two histologically confirmed CIN or CIN2+ lesions respectively, with a time interval of at least 18 months. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated.

Risk factors

Univariable and multivariable logistic regression models were performed to identify risk factors for CIN2+ within the IBD cohort. The following data from all women in PSI were collected: year of birth, IBD-type, age at time of diagnosis, Montreal classification[25]; for CD location (L) and behavior (B); for UC extension (E), smoking status, education level and exposure to immunosuppressive medication (immunomodulators and biologicals). Smoking was divided in current smoking and never or former smoking if patients withdrew within 6 months before inclusion in PSI. High education level was defined as having a college or university degree. Exposure to immunosuppressive medication was defined as at least one data entry of an immunomodulator (thiopurines, methotrexate) or a biologic agent (anti-TNFα, vedolizumab, ustekinumab) in PSI. Exposure was further subdivided in less or more than one year exposure. Risk factors with a significance level of <0.20 in univariable analyses were taken into account in the multivariable analysis.

Coverage for cervical testing

All women living in the Netherlands receive an invitation to participate in the national cervical cancer screening program every five years between ages 30 and 60 years.[26] Five year coverage rate for cervical smear testing was defined as the percentage of women within the screening age group that had at least one cervical test in the five years before the reference date, either within the organized screening program or outside of the programme (e.g. by indication). Adherence to the national cervical cancer screening program was defined as at least one primary test performed within the program. For 5-year coverage rates, periods of five consecutive years were analyzed. For example: the coverage rate of 2016 is based on tests performed in the 2012-2016 period for women born between 1952 and 1986. Our results were compared with data from the nationwide monitoring of the National cervical cancer screening program.[24]

Ethical Approval

All patients in the PSI-IBD dataset provided written informed consent. The scientific boards of the Dutch IBD biobank and PALGA approved the study. The ethics committees of all eight participating UMCs granted permission to link study objects from the PSI cohort to their own cervical records collected in PALGA under strict privacy procedures. Consent by women for the use of their data stored in PALGA is implicit according to the Dutch Ethical Code of reuse of data and PALGA's own privacy policy.

RESULTS

Study population

A total of 2,098 IBD women (median age at inclusion 42 years) were included. The matched cohort comprised 8,392 women. Median follow-up was 13 years in both cohorts (range 0-16 years). The IBD cohort comprised 1,382 (66%) patients with CD and 716 (34%) patients with UC, IBD-unclassified (IBD-U) or IBD-indeterminate (IBD-I). Within the IBD cohort, 554 (26.4%) women were smokers and 461 (34.6%) had a high education level. A total of 1,030 (49%) patients were exposed to immunomodulators and 707 (34%) to biologic agents **(Table 1).** CD patients were more often smokers (33.8% vs 15.0%, P: < 0.001) and were more often exposed to immunosuppresssants (immunomodulators 53.0 % vs 41.7%, biologicals 42.2% vs 16.9%, P: <0.001) than UC patients **(Supplementary Table 1).** Number of screening episodes in a 5 year period was significantly higher in the IBD cohort than in the matched cohort, 30% in the IBD cohort had more than one screening episode in a 5 year period, compared to 20.9% in the matched cohort (P: <0.001) **(Table 1)**.

Standardized detection rates

Over the whole study period, significantly more CIN2+ lesions were detected in the IBD cohort compared to the matched cohort (SDR 1.27, 95% CI 1.05-1.52). This difference was mainly due to more CIN2 lesions in the 35 to 39 years of age group. No differences were observed in

detection rates of CIN1 lesions (SDR 0.95, 95% CI 0.68-1.37) CIN3 lesions (SDR 1.21, 95% CI 0.94-1.55) or cervical cancer (SDR 0.30, 95% CI 0.03-1.08) (**Table 2**). Significant more CIN2+ lesions were detected in the 2006-2010 time period. Urbanization was not a strong influencing factor for detecting CIN2+ (**Table 2**).

Incidence rate of CIN2+ during longitudinal follow-up

The risk of progression of a normal smear towards CIN2+ was higher in IBD women than in women from the matched cohort. After exclusion of women with an abnormal smear at first available cytopathology record, the risk of developing a CIN2+ lesion was significantly higher in the IBD cohort; incidence rate ratio (IRR) for CIN2+ for IBD women was 1.66 (95% CI 1.21-2.25) compared to the matched cohort. This was due to an increased risk of CIN2 (IRR 1.83, 95% CI 1.15-2.91) and CIN3 (IRR 1.56, 95% CI 1.01-2.41), not cervical cancer (IRR 1.14, 95% CI 0.16-5.13).

Table 1. Patient demographics from PSI for IBD women and screening behavior for IBD and matched women

		IBD women
Total number of women		**2 098**
Diagnosis	CD	1,382 (65.9)
	UC, IBDU or IBDI	716 (34.1)
Age at IBD diagnosis	<25 years	772 (36.0)
	≥25 years	1,321 (63.0)
	N/A	5 (0.2)
Smoking status[a]	Never/>6 months	1,466 (70.9)
	Current/<6 months	554 (26.4)
	N/A	78 (3.7)
Education level[b]	Low	1,352 (64.4)
	High	700 (33.4)
	N/A	46 (2.2)
Medication exposure[c]		
Immunomodulator	No	1068 (50.9)
	< 1 year	237 (11.3)
	> 1 year	793 (37.8)
Biologicals	No	1391 (66.3)
	< 1 year	227 (10.8)
	> 1 year	480 (22.9)
Crohn's disease		
Montreal L	L1	256 (18.5)
	L2	277 (20.0)
	L3	530 (38.4)
	L4 or L1-3 + L4	155 (11.2)
	N/A	164 (11.9)
Montreal B	B1	495 (35.8)
	B2	191 (13.8)
	B3	192 (13.9)
	B1-3 + p	347 (25.1)
	N/A	157 (11.4)
Ulcerative colitis		
Montreal E	E1	56 (7.8)
	E2	238 (33.2)
	E3	346 (48.3)
	N/A	76 (10.3)

Table 1. Continued.

		IBD women	Matched women	*P value*
Total number of women		**2 098**	**8 379**	
Total number of screening episodes		6 654	23 344	
Number of screening episodes per woman in a 5 year period				
	1	1,451 (69.2)	6,595 (78.7)	<0.001
	>1	567 (27.0)	1,646 (19.6)	
	>2	80 (3.0)	138 (1.3)	
Urbanization level	>100 000	632 (30.1)	2516 (30)	0.931
	<100 000	1466 (69.9)	5863 (70)	

Values are expressed as total number (n) and %. [a] Smoking was defined as current smoker or former smokers who quitted within 6 months prior to inclusion in PSI. [b] High defined as having a college or university degree. [c] Exposure to medication use was defined as at least one data entry of an immunomodulator (thiopurines, methotrexate) or a biological (anti-TNFα, vedolizumab) in the database. Abbreviations: IBD: inflammatory bowel disease, PSI: Parelsnoer Institute, N: number; IQR interquartile range, CD: Crohn's disease, UC: ulcerative colitis, IBDU: IBD-unclassified, IBDI: IBD-indeterminate, N/A: not avaialable, L: location, B: behaviour, E: extent.

Table 2. Standardized detection ratios of cervical intraepithelial lesions and cervical cancer for IBD women by age, time period and urbanization, follow-up period 2000-2016 as compared to matched cohort

		CIN1[a]				CIN2[a]				CIN3[a]			
	No. prim. tests[b]	**Obs[b]**	**Exp[b]**	**SDR[b]**	**95% CI[c]**	**Obs[b]**	**Exp[b]**	**SDR[b]**	**95% CI[c]**	**Obs[b]**	**Exp[b]**	**SDR[b]**	**95% CI[c]**
Overall detection rate[b]	6 654	35	35.6	0.98	0.68-1.37	52	34	**1.53**	**1.14-2.01**	64	52.7	1.21	0.94-1.55
Screening age													
<29	348	7	7.3	0.96	0.38-1.98	7	8.3	0.84	0.34-1.74	5	8.0	0.63	0.20-1.46
29-34	1,457	11	6.4	1.72	0.86-3.08	15	11.7	1.28	0.71-2.12	25	21.7	1.15	0.75-1.70
35-39	1,068	3	7.1	0.42	0.09-1.24	11	4.2	**2.62**	**1.31-4.69**	11	7.9	1.39	0.69-2.49
40-44	1,136	9	6.0	1.50	0.68-2.85	10	4.3	**2.33**	**1.11-4.28**	7	5.7	1.23	0.49-2.53
45-49	1,060	2	4.4	0.45	0.05-1.64	5	2.4	2.08	0.67-4.86	8	4.2	1.91	0.82-3.75
50-54	706	0	2.0			1	1.5	0.67	0.02-3.72	5	2.1	1.91	0.51-4.88
55-59	594	3	1.7	1.77	0.36-5.16	3	0.9	3.33	0.67-9.74	2	1.2	1.67	0.20-6.02
≥60	285	0	0			0	0			1	0.6	1.67	0.02-9.27
Total	6,654	35	35.0	1.00	0.70-1.39	52	33	**1.57**	**1.17-2.05**	64	51.4	1.25	0.96-1.59
Time period													
2000-2005	2,157	5	9.3	0.54	0.17-1.26	12	7.9	1.52	0.78-2.65	19	17.3	1.10	0.66-1.72
2006-2010	2,006	15	11.1	1.35	0.76-2.23	19	8.7	**2.18**	**1.31-3.41**	19	16.0	1.19	0.71-1.85
2011-2016	2,491	15	15.4	0.97	0.54-1.61	21	17.7	1.19	0.73-1.81	26	17.4	1.49	0.98-2.19
Total	6,654	35	35.8	0.98	0.68-1.36	52	34.3	**1.51**	**1.13-1.98**	64	50.7	1.26	0.97-1.61
Urbanization													
High level	1,962	9	13.3	0.68	0.31-1.29	17	15.4	1.10	0.64-1.77	24	17.2	1.40	0.89-2.08
Low level	4,692	26	22.4	1.16	0.76-1.70	35	23.0	**1.52**	**1.06-2.12**	40	31.7	1.26	0.90-1.72
Total	6,654	35	35.8	0.98	0.68-1.36	52	38.4	**1.35**	**1.01-1.78**	64	48.9	**1.31**	**1.01-1.67**

Table 2 Continued. Standardized detection ratios of cervical intraepithelial lesions and cervical cancer for IBD women by age, time period and urbanization, follow-up period 2000-2016 as compared to matched cohort

		Cervical cancer[a]				CIN2+[a]			
	No. prim. tests[b]	Obs[b]	Exp[b]	SDR[b]	95% CI[c]	Obs[b]	Exp[b]	SDR[b]	95% CI[c]
Overall detection rate[b]	6 654	2	6.7	0.30	0.03-1.08	**118**	**93.2**	**1.27**	**1.05-1.52**
Screening age									
<29	348	0	0.3			12	16.7	0.72	0.37-1.26
29-34	1 457	0	1.5			40	35.0	1.14	0.82-1.56
35-39	1 068	1	1.1	0.91	0.01-5.06	23	12.8	**1.80**	**1.14-2.70**
40-44	1 136	0	0			17	10.2	1.67	0.97-2.67
45-49	1 060	1	1.1	0.91	0.01-5.06	14	8.5	1.65	0.90-2.76
50-54	706	0	0.7			6	4.2	1.42	0.52-3.11
55-59	594	0	0.6			5	2.4	2.08	0.68-4.86
≥60	285	0	0.3			1	0.9	1.11	0.03-6.19
Total	6 654	2	5.5	0.36	0.04-1.31	118	90.7	**1.30**	**1.08-1.56**
Time period									
2000-2005	2 157	0	2.2			31	25.5	1.22	0.83-1.73
2006-2010	2 006	0	2.0			38	25.7	**1.48**	**1.05-2.03**
2011-2016	2,491	2	2.5	0.80	0.10-2.89	49	37.1	1.32	0.98-1.75
Total	6 654	2	6.6	0.30	0.03-1.10	118	89.5	**1.26**	**1.04-1.51**
Urbanization									
High level	1 962	2	0.6			43	33.4	1.29	0.93-1.73
Low level	4 692	0	4.7			75	61.0	1.23	0.97-1.54
Total	6 654	2	5.3	0.38	0.04-1.37	118	94.4	**1.25**	**1.04-1.50**

[a] CIN: Cervical intraepithelial neoplasia; CIN1: mild dysplasia; CIN2: moderate dysplasia; CIN3: severe dysplasia or carcinoma in situ; cervical cancer: invasive cervical squamous cell carcinoma and non-clear cell adenocarcinoma; CIN2+: CIN2 or worse [b] No. of prim tests: number of primary screening tests; Detection rate is the percentage of episodes starting with a primary cytology or histology screen test resulting in a histological diagnosis of CIN or cervical cancer. Obs: detection rate in the IBD cohort. Exp: detection rate in the age and year of screening matched cohort. SDR Standardized detection ratio: defined as observed detection rate in IBD cohort compared to the expected detection rate. [c] 95% CI: 95% confidence interval based on a Poisson distribution. **Bold** numbers: statistically different.

No difference was observed in women developing CIN1 as worst histological diagnosis (IRR 0.95, 95% CI 0.57-1.60) **(Supplementary Table 2, Figure 1A-B)**. The cumulative incidence for CIN2+ as worst histological diagnosis during follow-up increased with age **(Figure 1C).**

Including women with prevalent lesions at the first available cytopathology record resulted in a lower IRRs but still a significantly higher CIN2+ risk for IBD women (IRR 1.37, 95%CI 1.10-1.70) **(Supplementary Table 2, Supplementary Figure A-C)**. After correcting for screening behavior and urbanization in a Cox proportional hazards model, CIN2+ risk in IBD women was also increased (HR 1.46, 95% CI 1.07-2.00) **(Supplementary Table 3)**.

Persistent or recurrent CIN lesions

In the IBD cohort an increased risk of persistent or recurrent CIN lesions was observed. A total of 17 (0.8%) IBD women had persistent CIN lesions during follow-up, compared to 36 (0.4%) in the matched cohort (OR 1.89, 95% CI 1.06-3.38, p 0.028). A total of 11 (0.5%) IBD women had persistent CIN2+ lesions during follow-up, compared to 15 (0.2%) in the matched cohort (OR 2.94, 95% CI 1.08-6.1, p 0.004).

Risk factors for CIN2+ in the IBD cohort.

In multivariable analysis, CIN2+ risk was associated with ileocolonic (L3) and/or upper-GI (L4) location in women with CD (adjusted OR 1.84, 95% CI 1.05-3.24), smoking (adjusted OR 3.20, 95% CI 1.90-5.40) and more than one or two screening episodes within a five-year period (adjusted OR 2.00, 95% CI 1.16-3.44 and 5.02, 95% CI 1.89-13.35) respectively). Exposure to immunomodulators or biologic agents was not associated with CIN2+ risk **(Table 3).**

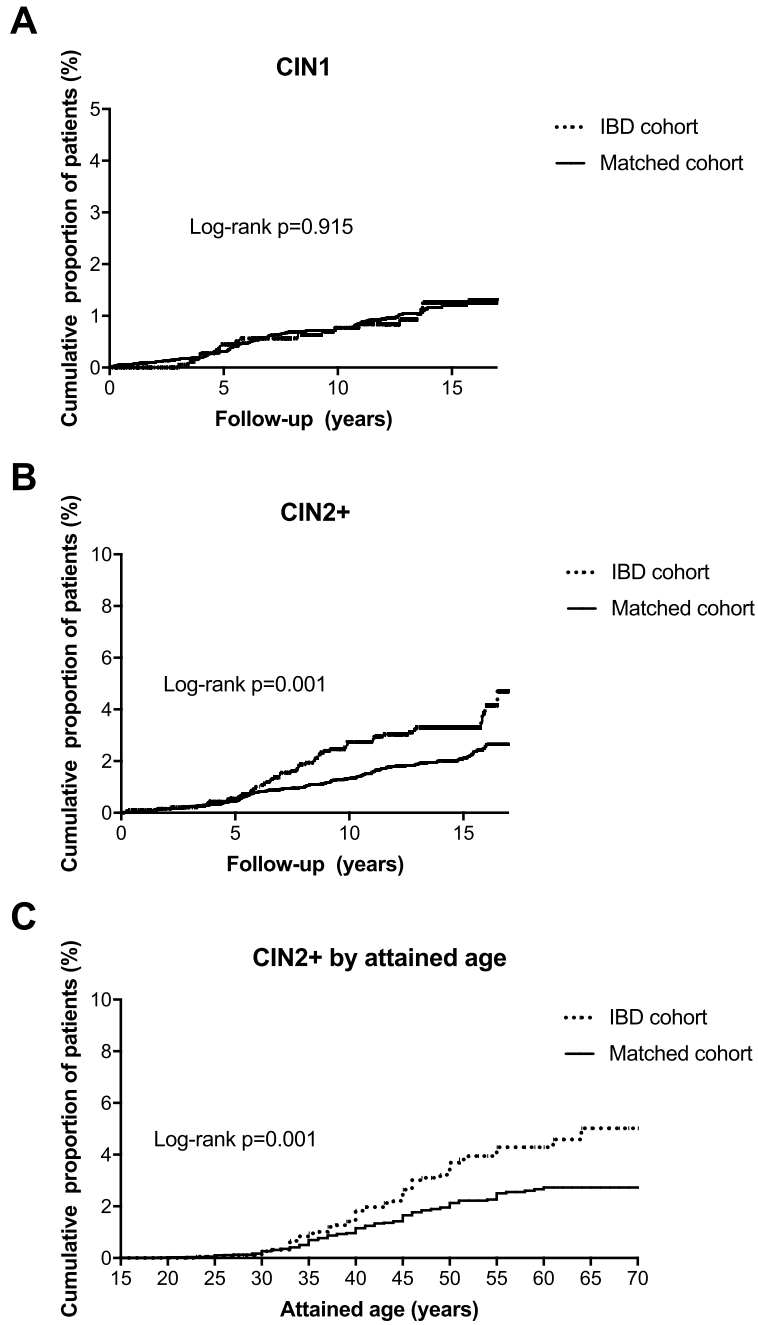

Figure 1A-C. Cumulative incidence of CIN estimates by Kaplan Meier as worst diagnosis for the IBD cohort and matched cohort by years of follow-up for CIN1 (A), CIN2+ (B) and by CIN2+ attained age (C), excluding women with a primary abnormal screen. Attained age is defined as the age at diagnosis of CIN2+ or age at end of follow-up.

Table 3. Covariates associated with a CIN2+ diagnosis in 2000-2016 for women with IBD

	Univariate analysis		Multivariable analysis	
	OR	**95%CI**	**OR**	**95%CI**
Screening episodes in a 5 year period				
1 episode	1.00	Ref	1.00	Ref
>1 episode	1.64	1.08-2.48	2.00	1.16-3.44
>2 episodes	3.26	1.60-6.62	5.02	1.89-13.35
Urbanization				
Low level	1.00	Ref	1.00	
High level	1.43	0.96-2.13	1.41	0.81-2.44
Disease type				
UC	1.00	Ref	1.00	1.00
CD	1.39	0.90-2.13	0.96	0.61-1.53
Age at diagnosis				
≥25 years	1.00	Ref	1.00	Ref
<25 years	1.60	1.09-2.36	1.54	0.91-2.59
CD behaviour				
B1	1.00	Ref		
B2, B3 or all p	0.79	0.49-1.26		
CD location				
L1 or L2	1.00	Ref	1.00	Ref
L3 or all L4	1.92	1.14-3.24	1.84	1.05-3.24
UC extent				
E1 or E2	1.00	Ref		
E3	0.67	0.31-1.45		
Education level				
Low	1.00	Ref	1.00	Ref
High	0.77	0.51-1.15	0.63	0.37-1.09
Smoking status				
No	1.00	Ref	1.00	Ref
Yes	2.59	1.74-3.86	3.20	1.90-5.40
Exposure to immunomodulators				
No	1.00	Ref	1.00	Ref
< 1 year	0.42	0.18-0.99	0.37	0.13-1.09
> 1 year	0.89	0.59-1.33	0.91	0.54-1.55
Exposure to biologicals				
No	1.00	Ref		
< 1 year	0.72	0.36-1.47		
> 1 year	0.96	0.61-1.54		

Abbreviations: CIN: Cervical intraepithelial neoplasia. CIN2+: CIN2 or worse; IBD: inflammatory bowel disease. OR: Odds ratio, CI: confidence interval; CD: Crohn's disease; B: behavior, L: location; UC: ulcerative colitis, E: extent

Screening adherence

IBD women participated consistently less often in the national cervical cancer screening program than women from the general population and their adherence rate declined over the years 2010 to 2016 (66.6% to 64.5%), similar to the general population (69.6% to 67.2%). The five-year coverage rate for cervical smear testing declined strikingly from 82.7% in 2012 to 74.2% in 2016 for IBD women. This was merely explained by a decline of cervical testing for other indications, i.e. outside the national cervical cancer screening program. Cervical screening outside the national program was more often performed in the IBD cohort than in the matched cohort, but declined from 16.8% in the 2008-2012 period to 9.7% in the 2012-2016 period **(Table 4).**

Table 4. Five year coverage rate of cervical smear testing from 2010 to 2016 in percentages for IBD women compared to women from general population

Year	**Total cervical screening**		**National cervical cancer screening program**		**Opportunistic cervical screening[a]**	
	IBD	General population	IBD	General population	IBD	General population
2010	76.7%	79.0%	66.6%	69.6%	10.1%	9.4%
2011	77.5%	77.8%	66.7%	68.4%	10.8%	9.4%
2012	82.7%	75.3%	65.9%	66.9%	16.8%	8.5%
2013	75.2%	75.6%	63.8%	67.2%	10.4%	8.5%
2014	76.7%	75.5%	65.4%	67.2%	11.3%	8.4%
2015	74.8%	75.3%	64.3%	67.3%	10.5%	8.2%
2016	74.2%	75.1%	64.5%	67.2%	9.7%	8.0%

Abbreviations: IBD: inflammatory bowel disease. [a] Cervical screening outside of the national screening program, indicative or secondary tests only

DISCUSSION

Results from our case-controlled cohort study show a higher detection rate of CIN2+ lesions in IBD women than in matched women from the general population. According to current guidelines, these lesions require treatment in most cases.[27] The difference in CIN2+ detection rate was highest in IBD women between the age of 35 and 39 years. The detection rate of cervical cancer was not significantly different between the two groups, probably due to the sample size. Even after correcting for their screening behavior, IBD women were still at increased risk of CIN2 and CIN3 lesions during follow-up. Also, after excluding all women with prevalent CIN lesions at the first screen, the risk for CIN2+ remained increased. Risk factors associated with CIN2+ in IBD women were smoking and ileocolonic (L3) and/or upper-GI (L4) location. Exposure to immunosuppressive medication was not identified as a risk factor.

Our study supports previous observations that IBD women are at increased risk of high-grade CIN.[16, 28] In addition to previous data, we have shown that during longitudinal follow-up, women with IBD show a higher rate of progression from normal smears to CIN2+ and

more often have persistent or recurrent CIN lesions than women in the general population. A higher rate of persistence of an hrHPV infection might explain for both findings. Transient and productive HrHPV infections and cytological low-grade abnormal smears, histologically mostly classified as CIN1, are highly prevalent and known to clear or regress spontaneously in many patients, especially in young women.[5,27] However, as opposed to transient or productive hrHPV infections, it is persistent or transforming infections that are essential in carcinogenesis.[5,29,30] Since there was no difference in CIN1 detection between our two groups, the acquisition of transient infections was probably not increased. Further data on persistence of hrHPV in IBD women and the role in cervical carcinogenesis are required, and may lead to strategies of (temporal) reduction of immunosuppressant use to clear the virus and halt, or even reverse cervical carcinogenesis, before passing a point of no return.

In our IBD cohort, ileocolonic (L3) or upper-GI (L4) location in women with Crohn's disease and smoking were risk factors for CIN2+ in multivariable analysis, whereas exposure to immunosuppressants was not associated with CIN2+. Onset of IBD before the age of 25 was a risk factor in univariable analysis only. Although younger age at IBD onset has already been identified as a risk factor[9], increased risk by disease location in Crohn's disease is a novel finding. Both young age at IBD onset and L3 or L4 disease location may be associated with a severe phenotype, might increase risk for CIN lesions, since chronic systemic inflammation can impair innate and adaptive cellular immune responses and may therefore result in a decreased clearance of hrHPV.[31] Studies on immunosuppressive medication as a risk factor for CIN and cervical cancer in IBD patients display discordant results. Some studies have previously found a significant association[8-11,15,32], while others have not.[7,13,14] In our study exposure to immunomodulators and biologics were studied as trichotomous variables. Further studies are needed to scrutinize the exact role for immunosuppressive medication in cervical neoplasia risk, split on duration of exposure, age of start, combination therapy and use of corticosteroids. Smoking was strongly associated with CIN2+ in our IBD cohort. This is consistent with previous findings, both in the general population[33, 34] and amongst women with IBD.[8,14] In our IBD cohort the risk of CIN2+ in active smokers was higher (adjusted OR 2.89, 95% CI 1.89-4.41) than the estimated 2-fold risk of CIN2+ in ever smokers in the general population[34-36], suggesting a combined effect of IBD and exposure to cigarette smoke.

IBD women had a higher screening frequency than women from the general population, as shown by the number of screening episodes within a five-year period. This might be explained by the fact that IBD women are referred to a gynecologist more often or are more aware of the increased risk and request intensified screening. This more frequent screening behavior could easily have influenced the incidence rate of CIN2+ in our study population. Undeniably, an increased number of cervical smears per individual increases the chance of detecting abnormalities. However, the hazard ratio for acquiring CIN2+ was still higher in the IBD cohort than in the matched cohort after correcting for this important confounder in multivariable analysis.

This is one of the few studies reporting on screening behavior and adherence to a national cervical cancer screening program amongst IBD patients.[13,14,37,38] Current ECCO guidelines advice to improve the rate of adherence in IBD women, based on a study by Long et al showing a suboptimal rate of cervical smear testing in IBD patients.[16,37] Our study underlines this advice, especially since we observed a decline in screening rate over the past years, due to less frequent testing both within and outside of the national screening program.

Prevention of cervical neoplasia requires two important interventions. First, vaccination for HPV in all females up to 26 years of age, preferably before sexual activity, is recommended for all women as primary prevention strategy.[16] Normal immunogenic response to HPV vaccination has been reported in patients on immunosuppressive medication.[39] Data regarding efficacy in terms of decreasing incidence of cervical dysplasia in immunocompromised individuals are expected in the following years. Given the burden of other HPV-related (penile, oral and anal) cancers in men, vaccination in young males is also highly worth considering.[40,41] Next to that, secondary prevention by means of screening for premalignant cervical lesions within in a national cervical cancer screening program is advised. ECCO recommends for IBD women to follow European guidelines on cervical cancer screening for the general population[16, 42] and intensified screening approach for immunocompromised women. American guidelines also suggest intensified screening for IBD women using immunosuppressive medication, but not for all women with IBD.[17,18] This risk stratification is not fully substantiated by our data. A decision on an intensified screening program in IBD women requires careful consideration of burden to patients, costs and benefits. Based on available evidence, we recommend encouraging all IBD women to adhere to national cervical cancer screening programs and increased awareness among physicians is warranted.

Despite the novel longitudinal data presented in this nationwide cohort study, a few limitations of this study warrant consideration. Since our IBD cohort comprises only patients from tertiary referral centers, reflecting a population with more severe disease, results of this study might not be completely generalizable to all IBD patients.[43] Also, we did not have data on several other possible confounders such as sexual behavior and oral contraceptive use.[44] It has been shown that a higher proportion of women with inflammatory bowel disease have sexual dysfunction compared to matched controls.[45] Since sexual activity is a strong risk factor for CIN, it might be hypothesized that the association with IBD is even stronger.[33] Unfortunately, we were not able to draw conclusions on hrHPV status, since these data were only collected limitedly. Furthermore, we were not able to collect data from PALGA before the year 2000. Some women might have had a history of CIN before the index date of our follow-up period, which may have put them at higher risk of a subsequent lesion. Lastly, a group of women in the IBD cohort might have had a CIN2+ diagnosis before their IBD diagnosis. We did not exclude these women, based on the fact that IBD is a chronic disease that often starts years before the actual date of diagnosis. Moreover, since higher rates of cervical neoplasia were detected even to up to 10 years before IBD diagnosis[9], we believe including these women in the cohort was justified.

In conclusion, this study demonstrates that IBD is a risk factor for high-grade cervical neoplasia, especially in women who smoke and have a severe CD phenotype. Close surveillance of low-grade lesions and treatment of high-grade CIN is warranted given that persistent lesions were more prevalent in women with IBD, possibly reflecting a decreased clearance of hrHPV. Vaccination for HPV and adherence to cervical cancer screening programs should be strongly encouraged in all IBD women, regardless of immunosuppressant use.

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SUPPLEMENTARY DATA

Supplementary Table 1. Patient demographics of women with Crohn's disease and ulcerative colitis

		CD n (%)	**UC, IBDU/I** n (%)	***P* value**
Total number of women		**1 382 (65.9)**	**716 (34.1)**	
Age at IBD diagnosis	<25 years	586 (42.4)	186 (26.1)	<0.001^
N/A for 5 (0.2%)	≥25 years	795 (57.6)	526 (73.9)	
Montreal L	L1	256 (21.0)		
N/A for 164 (11.9%)	L2	277 (22.7)		
	L3	530 (43.5)		
	L4	6 (0.5)		
	L1+L4	28 (2.3)		
	L2+L4	30 (2.5)		
	L3+L4	91 (7.5)		
Montreal B	B1	495 (40.4)		
N/A for 157 (11.4%)	B2	191 (15.6)		
	B3	192 (15.7)		
	B1p	123 (10.0)		
	B2p	60 (4.8)		
	B3p	164 (13.4)		
Montreal E	E1		56 (8.8)	
N/A for 76 (10.6%)	E2		238 (37.2)	
	E3		346 (54.1)	
Smoking status	Never	882 (66.2)	584 (85.0)	<0.001^
N/A for 78 (3.7%)	Current/<6 months	451 (33.8)	103 (15.0)	
Education level				
N/A for 46 (2.2%)	Low	911 (65.4)	472 (66.4)	0.777^
	High	461 (34.6)	239 (33.6)	
Medication exposure				
N/A for 33 (1.6%)				
Immunomodulator	No	650 (47.0)	418 (58.4)	
	<1 year	170 (12.3)	67 (9.4)	
	>1 year	562 (40.7)	231 (32.3)	<0.001^
Biological	No	796 (57.6)	595 (83.1)	
	<1 year	174 (12.6)	53 (7.4)	
	>1 year	412 (29.8)	68 (9.5)	<0.001^
Number of screening episodes in a 5 year period				
N/A for 38 (1.8%)	1	956 (69.2)	495 (69.1)	0.413^
	2	375 (27.2)	191 (26.7)	
	>2	50 (3.6)	30 (4.2)	

Abbreviations: IBD: inflammatory bowel disease, PSI: Parelsnoer Institute, N: number; yrs: years; IQR interquartile range, CD: Crohn's disease, UC: ulcerative colitis, IBDU: IBD-unclassified, IBDI: IBD-indeterminate, N/A: not applicable, L: location, B: behavior, E: extent, 5-ASA, 5-aminosalicylic acid * Mann-Whitney U test ^ Chi square test ** independent samples T-test

Supplementary Table 2. Observed number of CIN and cervical cancer cases, person-years, incidence rates per 1,000 person-years and incidence rate ratios for women with IBD compared to matched women from general population

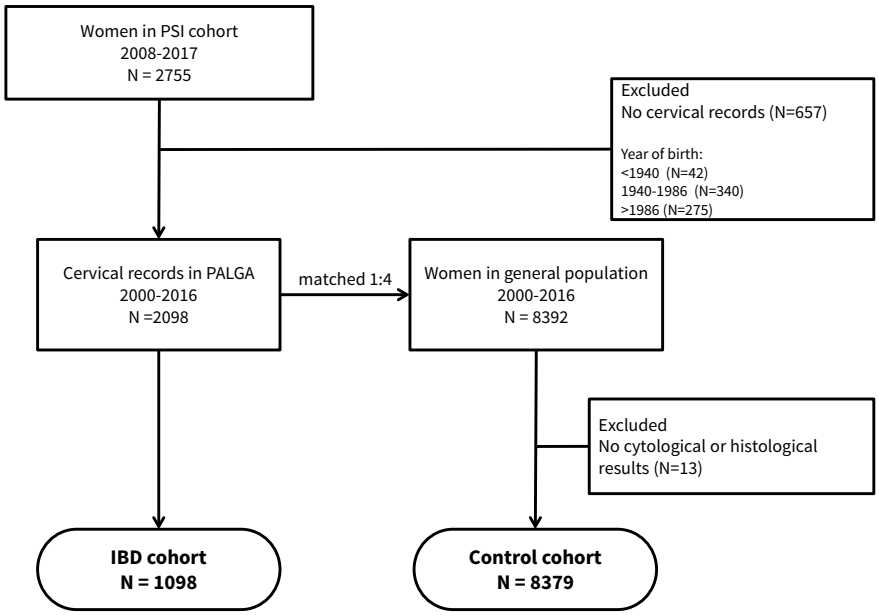
	Excluding women with an abnormal primary screen				**Including women with prevalent lesions**			
	Person-years	**Obs-No**	**IR (95% CI)**	**IRR (95% CI)**	**Person-years**	**Obs-No**	**IR (95% CI)**	**IRR (95% CI)**
CIN1								
IBD women	23,726	18	0.76 (0.45-1.20)	0.95 (0.57-1.60)	24,737	31	1.25 (0.85-1.80)	1.09 (0.73-1.61)
Matched women	92,956	74	0.80 (0.63-1.01)		98,730	114	1.16 (0.95-1.39)	
CIN2								
IBD women	23,235	26	1.12 (0.73-1.64)	**1.83 (1.15-2.91)**	24,624	46	1.87 (1.37-2.49)	**1.58 (1.12-2.22)**
Matched women	93,167	57	0.61 (0.46-0.79)		98,821	117	1.18 (0.98-1.42)	
CIN3								
IBD women	23,228	28	1.21 (0.80-1.74)	**1.56 (1.01-2.41)**	24,482	61	2.49 (1.91-3.20)	**1.34 (1.00-1.78)**
Matched women	93,030	72	0.77 (0.61-0.97)		98,083	183	1.86 (1.60-2.16)	
Cervical cancer								
IBD women	23,383	2	0.09 (0.01-0.28)	1.14 (0.16-5.13)	24,936	2	0.08 (0.01-0.27)	0.40 (0.06-1.47)
Matched women	93,381	7	0.07 (0.03-0.15)		99,406	20	0.20 (0.13-0.31)	
CIN2+								
IBD women	23,070	56	2.43 (1.83-3.15)	**1.66 (1.21-2.25)**	24,159	109	4.51 (3.71-5.44)	**1.37 (1.10-1.70)**
Matched women	92,726	136	1.47 (1.23-1.74)		97,163	320	3.29 (2.94-3.68)	

Abbreviations: CI: confidence interval; CIN, cervical intraepithelial neoplasia; CIN2+, CIN2, 3 or cervical cancer; IBD: inflammatory bowel disease. No. number; IR incidence rate; IRR incidence rate ratio

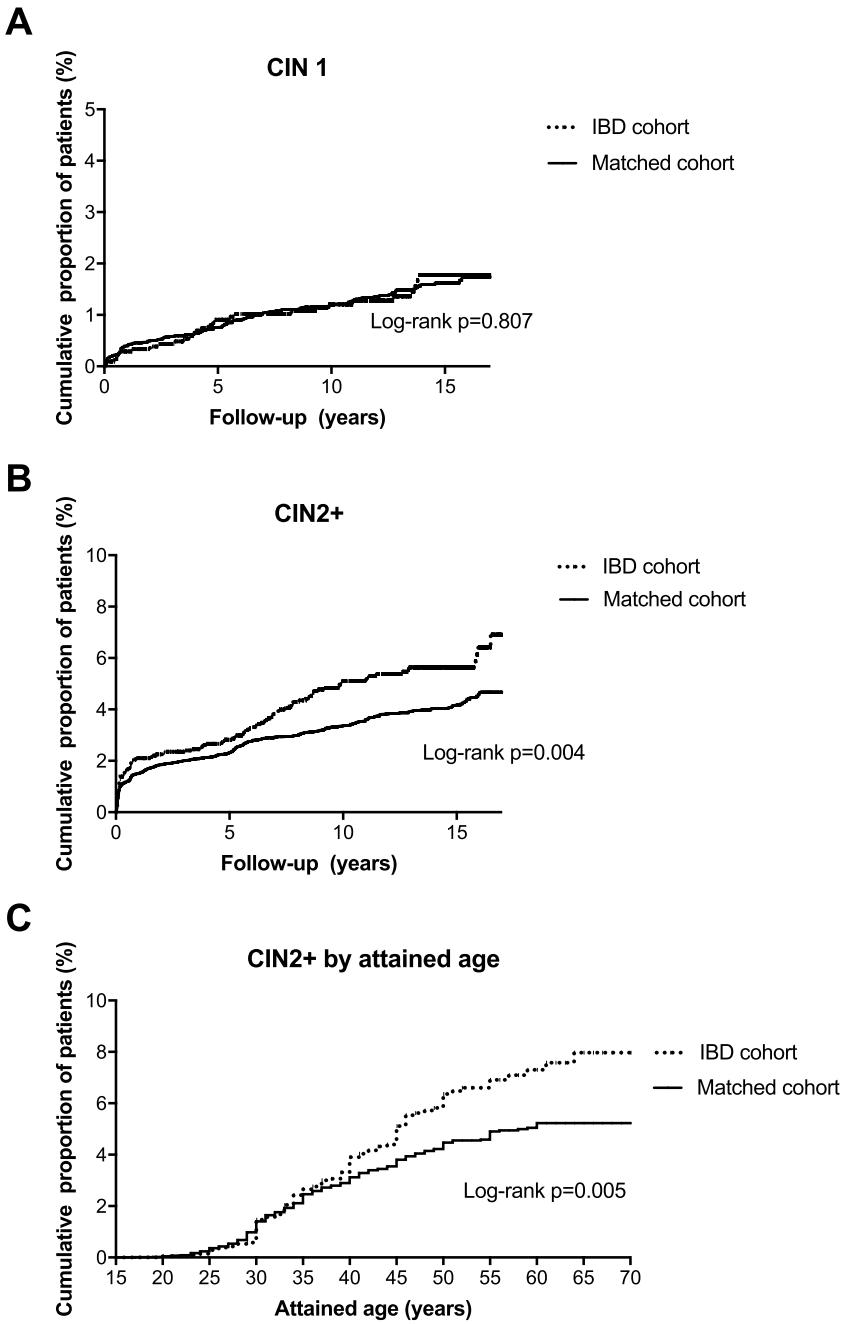
Supplementary Table 3. Univariable and multivariable Hazard ratios for different risk factors for CIN2+ over time in the total study population and after excluding women with a primary abnormal smear

	CIN2+ Excluding women with a primary abnormal screen				CIN2+ Total study population			
	Unavailable		Multivariable		Invariable		Multivariable	
	HR	95%CI	HR	95%CI	HR	95%CI	HR	95%CI
Case								
General population	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
IBD	1.66	1.21-2.26	1.46	1.07-2.00	1.37	1.10-1.70	1.28	1.03-1.60
Urbanization								
Low level	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
High level	1.08	0.79-1.47	1.11	0.81-1.51	1.31	1.07-1.60	1.33	1.09-1.62
Screening episodes in a 5 year period								
1 episode	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
1-2 episodes	1.74	1.27-2.38	1.68	1.23-2.30	1.31	1.05-1.63	1.28	1.03-1.60
>2 episodes	5.84	3.55-9.60	5.39	3.26-8.92	3.42	2.31-5.07	3.31	2.22-4.92

Abbreviations: CIN: Cervical intraepithelial neoplasia. CIN2+: CIN2, CIN 3 or cervical cancer; IBD: inflammatory bowel disease. HR: Hazard ratio, CI: confidence interval.



Supplementary Figure 1. Flowchart of study population



Supplementary Figure 2A-C. Cumulative incidence of CIN estimates by Kaplan Meier as worst diagnosis for the IBD cohort and matched cohort by years of follow-up for CIN1 (A), CIN2+ (B) and by CIN2+ attained age (C), for total cohort. Attained age is defined as the age at diagnosis of CIN2+ or age at end of follow-up.

CHAPTER 8

DRUG EXPOSURE AND CERVICAL NEOPLASIA IN WOMEN WITH INFLAMMATORY BOWEL DISEASE

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ABSTRACT

Background: Women with inflammatory bowel disease (IBD) are at increased risk of moderate to high-grade cervical intraepithelial neoplasia and cervical cancer (CIN2+). In this study, the association between cumulative exposure to immunomodulators (IM) and biologics (BIO) for IBD and CIN2+ was assessed.

Methods: Adult women diagnosed with IBD before December 31st 2016 in the Dutch IBD biobank and with available cervical cytological and/or histological data in the nationwide cytopathology database PALGA were identified. CIN2+ incidence rates in IM and BIO exposed patients were compared to unexposed patients and risk factors were assessed. Cumulative exposure to immunosuppressive drugs was evaluated in extended time-dependent Cox regression models. To account for latency of therapy effects exposure lag-time of 6 and 12 months was considered.

Results: The study cohort comprised 1981 IBD women, 99 (5%) developed CIN2+ during a median follow up of 17.2 years [IQR 11.0–25.6]. In total, 1305 (66%) women were exposed to immunosuppressive drugs (IM 58%, BIO 40%, IM and BIO 33%). CIN2+ risk increased per year exposure to IM (HR 1.15, 95% CI 1.08-1.24) or BIO (HR 1.15 95% CI 1.01-1.30), also after excluding the first 6 and 12 months of exposure. In multivariate analysis, smoking (HR 2.75, 95%CI 1.75-4.31) was a risk factor for CIN2+.

Conclusion: Cumulative exposure to IM and BIO is associated with increased risk of CIN2+ in IBD women. In addition to active counseling of IBD women to participate in cervical screening programs, further assessment of the benefit of intensified screening of IBD women on long-term exposure to IM or BIO is warranted.

INTRODUCTION

There is growing evidence that cervical neoplasia risk is increased in women with inflammatory bowel disease (IBD).[1-5] Infection with high-risk human papilloma virus (hrHPV) precedes virtually all cases of cervical intra-epithelial lesions (CIN) and cervical cancer. HPV not only initiates carcinogenesis but is probably also crucial for progression along the cascade of (pre) neoplastic lesions towards cervical cancer.[6-8] The carcinogenic potential of HPV infection is determined by HPV-type, persistence of HPV-infection and epigenetic alterations.[5, 9] It is hypothesized that IBD women who are exposed to HPV are at an increased risk of cervical neoplasia due to their chronic inflammatory state and frequent use of immunosuppressive drugs (IS).[5] Firstly, the (iatrogenic) immunocompromised state could result in persistence of HPV-infection (i.e. the inability to clear infection).[7, 10] Secondly, immunosuppression may be involved in induction of carcinogenesis and accelerated progression through pre-neoplastic stages due to impaired detection of oncogenic signals (immunosurveillance).[7, 11, 12]

The basis of the increased risk of cervical neoplasia in IBD has not been fully elucidated and the role of IS in this association is not well understood.[13-20] Recently, two Danish studies have reported an association between the risk of cervical neoplasia and treatment with anti-tumor-necrosis factor-alpha (anti-TNF), thiopurines and 5-aminosalicylic acid (5-ASA) for Crohn's disease.[16, 21] In contrast, Lees et al reported no association between the use of immunosuppressive therapy in IBD women and incidence of cervical neoplasia.[18] Longitudinal and detailed data on IS exposure are lacking to elucidate whether IBD medication increases the risk of cervical neoplasia.

Therefore, the aim of the current study was to assess the association of exposure to IS (immunomodulators (IM) and/or biologic agents (BIO)) and the development of moderate to high-grade cervical dysplasia and cervical cancer (CIN2+) in a national cohort of IBD women.

METHODS

Data resources

A cohort study using data from the Dutch IBD biobank and the Dutch nationwide network and registry of histology and cytopathology (PALGA) was performed. *The Dutch IBD biobank* is a nationwide collaborative bio-banking project of all eight University Medical Centers (UMCs, tertiary referrals centers) in the Netherlands, founded in 2007, in which data and biomaterial of adult IBD patients are prospectively and routinely collected. Every adult patient with an established diagnosis of IBD according to the Lennard-Jones criteria[22], treated in a UMC is eligible for inclusion. Diagnosis of IBD was confirmed by endoscopy, radiology and/or histology. After written informed consent, data are collected using a standardized information model containing 225 IBD-related items These items include patient demographics, disease

characteristics, radiographic imaging results, laboratory and endoscopy results, previous and current treatment characteristics. Thereafter, data are prospectively collected during patient visits.[23] The *Dutch nation-wide network and registry of histology and cytopathology (PALGA)* database stores all cervical histological and cytological results and has a nationwide coverage of all pathology labs from 1991 onwards.[24] Since 1996, all Dutch women aged 30-60 are invited for cervical smear examination every 5 years, within the national cervical cancer screening program. Women are identified by the first eight letters of their surname (maiden name is used for married women) and date of birth. This identification string enables the linkage of different tests in PALGA to an individual patient, and to follow individual testing histories (dates and diagnoses). The PALGA database also contains comprehensive information on the indication for assessment of cytology and/or histology (i.e. primary smear within the screening programme, opportunistic screening, follow-up screening test in case of abnormal smear according to the Dutch guidelines or smear of inadequate quality).[25] Coding of diagnostic information is based on the Systematized Nomenclature of Medicine (SNOMED) as published by the College of American Pathologists in 1982.[26] Pseudonymized data from the Dutch IBD biobank with a unique identifier for each participant was linked to the PALGA database.

Study population and data collection

Female patients of 17 years and older with IBD diagnosed before 31st of December 2016 identified in the Dutch IBD biobank with available cervical cytological and/or histological data in the PALGA database between 1st of January 2000 and 31st of 2016 were reviewed for eligibility. For all included IBD patients, detailed information on CIN, cancer and screening history was retrieved from the PALGA database. Patient demographics and clinical IBD data including disease characteristics, smoking habits, social economic status, history of malignancy, previous and current drug exposure were collected from the Dutch IBD biobank database.

Clinical outcome

The primary outcome of interest was the diagnosis of moderate to high-grade cervical intra-epithelial lesions and cervical cancer (CIN2+) in IBD women exposed to IS (IM and/or BIO) compared to IS-naive IBD women. Cervical intra-epithelial neoplasia (CIN) was defined as all histological neoplastic lesions in the cervix categorized as mild dysplasia (CIN1), moderate dysplasia (CIN2) and severe dysplasia or carcinoma in situ (CIN3), including glandular neoplasia (such as adenocarcinoma in situ (AIS)). Cervical cancer was classified into invasive squamous cell carcinoma and invasive adenocarcinoma. CIN2+ comprises CIN2, CIN3, AIS and invasive cervical cancer. Patients with more than one histology result during follow-up, had their outcome based on the first occurrence of the most severe (highest grade) histological diagnosis. Women with a history of any cancer except for non-melanoma skin cancer before the first recorded cervical smear were excluded. Women were also excluded if IBD was diagnosed after the last recorded cervical smear or after diagnosis of high-grade cervical dysplasia or cervical cancer (i.e. CIN2+). Follow up started from the date of IBD diagnosis (index date) and

continued until the first of the following study endpoints: highest grade of CIN2+ diagnosis, hysterectomy or December 31st, 2016.

Impact of immunosuppressive drugs

The IBD IS evaluated were IM (i.e. azathioprine (AZA), 6-mercaptopurine, thioguanine, methotrexate, tacrolimus and cyclosporine) and BIO (infliximab, adalimumab, certolizumab, golimumab, vedolizumab and ustekinumab). Patients with any documented use of IS following IBD diagnosis were considered as being 'exposed' to IS. Patients were considered as being exposed to both IM and BIO when treated with at least one IM and at least one BIO during follow up, either simultaneously or subsequently. Corticosteroids were not included in the analysis due to various indications, administration types and length of exposure.

Statistical analysis

Baseline demographics were expressed as median and interquartile ranges (IQR) or total count (n) and proportion (%). We compared demographic, disease related and treatment related factors, between IS exposed and non-exposed patients. To assess the association between IS exposure and CIN2+ risk two analyses were performed. In the first analysis the crude effect of exposed to IS *versus* non-exposed was assessed using incidence rates (IR) with 95% confidence intervals (95% CI) of CIN2+ lesions during follow-up. Risk factors for CIN2+ were assessed. Also population density was taken into account to correct for higher prevalence of cervical lesions in women living in urbanized areas, based on postal codes retrieved from the PALGA database. Postal code areas with < 100 000 inhabitants were considered low-density (rural) areas and postal code areas with >100 000 inhabitants were considered high-density areas (urban). These factors were expressed as mean and standard deviation (SD) or total count (n) and proportion (%). A p-value <0.05 was considered significant. In the second analysis, the effects of cumulative IM exposure and/or BIO exposure on CIN2+ risk were assessed as time-dependent variables in extended multivariable Cox regression models. For this analysis, all patients with detailed IS treatment documentation and non-exposed patients were included. Baseline was set at the date of IBD diagnosis and drug exposure was assumed to start on the first documented use in the IBD biobank. Cumulative exposure to IM and BIO were calculated separately, as 0.5 exposure years increase for each 6 months of active exposure, and the accumulated exposure remained stable during treatment pauses or after the end of treatment. Cumulative exposure was calculated until CIN2+ or end of follow up. The presence of effect modification between IM and BIO was evaluated by including an interaction term. Age at diagnosis, IBD type (CD or UC/IBDU) and smoking behavior, were considered potential confounders. These covariates were defined at cohort entry and included in the multivariable analyses as time-fixed variables. Statistical significance was defined as a p-value <0.05, all alternative hypotheses were 2-sided.

Sensitivity analyses

Cumulative drug exposure was lagged to reflect an expected latency period between drug exposure and onset of an effect, because CIN2+ is unlikely to develop instantly after exposure

to IS. Considering the uncertainty in the optimal length of the latency time window sensitivity analyses were conducted by varying the exposure time using lag periods of 6 and 12 months after the initial documented use. In the final time-dependent extended multivariable Cox regression analysis, 150 patients that were exposed to IS but with unknown duration of exposure were excluded, because multiple imputations would probably not yield a reliable estimation. Sensitivity analyses were conducted where these excluded patients were coded as *non-exposed* and *continuously exposed* without changing the overall outcome (**Supplementary Table 1**). All analyses were conducted using IBM SPSS Statistics version 25.1.0 (Armonk, NY, USA: IBM).

Ethical Approval

All patients in the PSI-IBD dataset provided written informed consent. The scientific boards of the Dutch IBD biobank and PALGA approved the study. The ethics committees of all eight participating UMCs granted permission to link study objects from the PSI cohort to their own cervical records collected in PALGA under strict privacy procedures. Consent by women for the use of their data stored in PALGA is implicit according to the Dutch Ethical Code of reuse of data and PALGA's own privacy policy.

RESULTS

Study population characteristics

In the PSI database, 2796 adult female patients were identified diagnosed with IBD before 31st of December 2016. A total of 834 patients were excluded, in the vast majority of cases due to unavailability of cervical data in the PALGA database (**Figure 1**). The study cohort included 1981 IBD women; 1318 (67%) were diagnosed with CD and 663 women (33%) were diagnosed with UC or IBD-U. The median follow-up time was 17.2 years [IQR 11.0 – 25.6 years]. Characteristics of the study population, overall and stratified by exposure to immunosuppressive treatment, are summarized in **Table 1**. During follow-up, 298 patients (15%) remained unexposed to *any* IBD drug. Among exposed patients, 1305 (67%) were exposed to treatment with an IS (IM and/or a BIO). Among these patients, 1155 (89%) received IM treatment, 795 (61%) received treatment with BIO, and 645 (49%) received treatment with IM and BIO either subsequently and/or simultaneously. At initiation of a second line of IS treatment, 58% of patients had been previously exposed to IM monotherapy and 24% to BIO monotherapy. Concomitant therapy with IM and BIO was the initial treatment in 18% of patients. Details on exposure to different IBD drug types are summarized in **Supplementary Table 3**. Women exposed to IS were significantly younger at IBD diagnosis than unexposed women ($p<0.001$). Also, exposed women had more ileo-colonic disease ($p<0.001$), upper GI disease ($p=0.035$), perianal involvement ($p<0.001$), extensive colitis ($p=0.003$) and a history of smoking ($p=0.008$) than non-exposed women (**Table 1**).

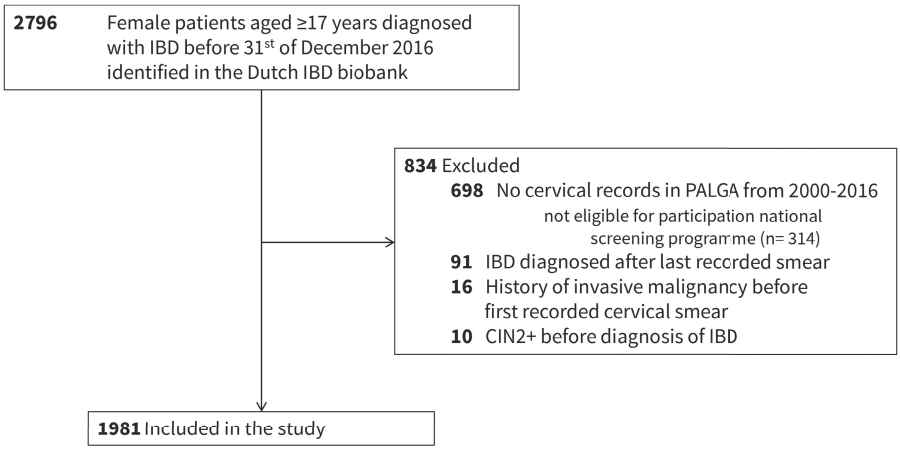

Figure 1. Flowchart of the study population

Table 1. Patient characteristics, overall and by exposure group

	Overall n=1981	Non-exposed to IS n=676	Exposed to IS n=1305	*P-value*
Age at inclusion in PSI (yrs), median (IQR)	42.0 [33.0-51.0]	46.5 [38.0-55.0]	40.0 [32.0-48.0]	0.152
Age at diagnosis (yrs), median (IQR)	27.8 [21.9-38.0]	30.0 [22.5-40.5]	27.0 [21.5-37.0]	<0.001
Diagnosis, n (%)				
Crohn's disease	1318 (66.5)	349 (51.6)	970 (74.3)	<0.001
UC/IBDU/IBDI	663 (33.5)	328 (48.4)	335 (25.7)	<0.001
CD – Montreal age at diagnosis, n (%)				
A1 <17 yr	101 (7.7)	29 (8.3)	72 (7.4)	0.239
A2 17-40 yr	964 (73.1)	245 (70.4)	719 (74.1)	<0.001
A3 >40 yr	253 (19.2)	74 (21.3)	179 (18.5)	0.080
CD – Montreal localization, n (%)	*n=1184*	*n=303*	*n=885*	
L1 Ileal	248 (20.1)	67 (22.7)	181 (20.5)	0.847
L2 Colonic	321 (27.1)	100 (33.3)	221 (25.0)	0.043
L3 Ileocolonic	572 (48.3)	123 (41.0)	449 (50.7)	0.001
L4 involvement	41 (3.5)	7 (2.3)	34 (3.8)	
Isolated upper GI disease	13 (1.1)	3 (1.0)	10 (1.2)	0.035
Perianal involvement	346 (29.2)	72 (24.0)	274 (31.0)	<0.001
Isololated perianal disease	30 (2.5)	6 (2.0)	24 (2.7)	
CD – Montreal disease behavior, n (%)	*n=1172*	*n=306*	*n=860*	
B1 Non-stricturing, non-penetrating	590 (50.3)	168 (54.9)	417 (48.5)	0.048
B2 Stricturing	239 (120.4)	54 (17.6)	185 (21.5)	0.144
B3 Penetrating	343 (29.3)	84 (27.5)	258 (30.0)	0.383

Table 1. Continued.

	Overall n=1981	Non-exposed to IS n=676	Exposed to IS n=1305	*P-value*
UC – Montreal disease extent	*n=574*	*n=283*	*n=291*	
E1 Proctitis	43 (7.5)	31 (11.0)	12 (4.1)	0.002
E2 Left-sided	208 (36.2)	110 (38.9)	98 (33.7)	0.152
E3 Extensive colitis	323 (56.3)	142 (50.2)	181 (62.2)	0.003
Smoking history	*n=1906*	*n=653*	*n=1233*	
Never	728 (38.2)	277 (42.4)	445 (36.1)	0.008
Current	522 (27.4)	140 (21.6)	417 (33.8)	<0.001
Former (>6 months abstinent)	656 (34.4)	236 (36.1)	371 (30.1)	0.002
Pharmacological IBD treatment				
Exposure to any IBD drug	1683 (85.0)	378 (55.8)	1305 (100)	-
5ASA	1119 (56.5)	338 (49.9)	784 (60.1)	<0.001
Cortiosteroids	1006 (50.7)	196 (29.0)	810 (62.1)	<0.001
Immunomodulator	1155 (58.3)	0 (0)	1155 (88.5)	-
Biologics	795 (40.1)	0 (0)	795 (60.9)	-
Immunomodulator or biologic	1305 (65.9)	0 (0)	1305 (100)	-
Immunomodulator and biologic	645 (32.6)	0 (0)	645 (49.4)	-
Duration therapy, months (median, IQR)				
Immunomodulator (n=1005)	42 [12-78]			
Biologic agent (n=696)	36 [12-66]			

Yrs, years; IQR, interquartile range; CD, Crohn's Disease; UC, ulcerative colitis; IBDU, IBD-unclassified; IBDI, IBD-indeterminate; 5-ASA, 5-Aminosalicylic acid.
Data are reported as median with interquartile range.

Risk factors for CIN2+

Overall, 99 cases of CIN2+ occurred between 1st January 2000 and 31st December 2016 (IR 2.73 per 1000 PY, 95% CI 2.23 – 3.31). Median age at diagnosis of CIN2+ was 36.6 years [IQR 31.6 - 45.8]. Among patients with CIN2+, 44 developed CIN2 lesions (44%), 53 developed CIN3 lesions (54%) and 2 patients developed cervical cancer (2%). Patients with CIN2+ lesions more often had a history of smoking than patients without CIN2+ (71% vs. 59%, p=0.006). Also, CIN2+ patients were younger (45.2 years vs. 49.1 years, p=0.012) than patients without CIN2+ **(Table 2).**

Table 2. Factors associated with CIN2+ lesions

	CIN2+ (n=99)	**no CIN2+ (1882)**	**p-value**
Crohn's Disease	74 (74.5)	1244 (66.1)	0.08
Mean age at diagnosis (SD)	27.8 (10.2)	30.8 (12.2)	0.062
Mean age (SD)	45.3 (9.9)	49.1 (11.9)	0.010
Perianal involvement	22 (22.4)	324 (17.2)	0.201
Montreal E3	12 (12.1)	311 (16.7)	0.97
Montreal B2/B3	28 (28.3)	554 (29.7)	0.576
Montreal L3	41 (41.4)	530 (28.4)	0.085
Urban population density	33 (33.7)	561 (29.8)	0.456
5ASA exposure (n=1105)	49 (55.1)	1070 (56.4)	0.150
Steroid exposure (n=1006)	54 (55.1)	952 (50.9)	0.406
Exposure IM (n=1155)	57 (57.1)	1098 (58.3)	0.880

Table 2. Continued.

	CIN2+ (n=99)	no CIN2+ (1882)	p-value
Exposure BIO (n=795)	41 (41.8)	754 (39.8)	0.789
Exposure to any IBD drug (n=1662)	82 (82.7)	1601 (84.9)	0.543
Unexposed to any IBD drug (n=124)	2 (2.0)	122 (6.5)	0.074
Exposure to IS (n=1305)	68 (68.4)	1237 (65.3)	0.545
Exposure to IM AND BIO (n=645)	30 (30.6)	615 (32.7)	0.623
Current smoking	46 (46.9)	476 (25.0)	<0.0001
Ever smoking	70 (71.4)	1108 (58.7)	0.029

SD, standard deviation; 5-ASA, 5-Aminosalicylic acid; IS, immunosuppressive drugs; IM immunomodulators; BIO, biological.

Impact of immunosuppressive treatment

Sixty-eight cases of CIN2+ occurred in IBD patients that were exposed to IS (IR 3.03, 95% CI 2.37-3.82) and 31 cases in patients never exposed to IS (IR 2.23, 95% CI 1.57-3.18) (**Table 3**). Overall, IBD women exposed to IS did not have a higher risk of CIN2+ than non-exposed IBD women. For the analysis of cumulative immunosuppressive exposure, 1831/ 1981 (92.4%) IBD women with a detailed database record of medication use were included. Among them, 686 women (37.5%) remained non-exposed to IS, 1002 (54.7%) women were exposed to IM and 696 (38.0%) women were exposed to BIO. Exposure to IM and BIO was documented in 553 patients (30.2%); 225 (12.3%) received these drugs sequentially and 328 (17.9%) patients received combination treatment at some point (**Supplementary Table 4).**

Table 3. Incidence of cervical neoplasia according to treatment group

	Overall (n=1981) (36279 PY)		Unexposed to IS (13876 PY)		Exposed to IS (22403 PY)	
	No of events	IR per 1000PY (95% CI)	No of events	IR per 1000PY (95% CI)	No of events	IR per 1000PY (95% CI)
All CIN2+	99	2.73 (2.23 - 3.31)	31	2.23 (1.57 - 3.18)	68	3.03 (2.37 - 3.82)
Cervical cancer	2	0.06 (0.01 - 0.22)	1	0.07 (0.01 - 0.51)	1	0.04 (0.01 - 0.32)
CIN3	53	1.46 (1.11 - 1.91)	20	1.44 (0.93 - 2.23)	33	1.47 (1.05 - 2.01)
CIN2	44	1.18 (0.89 - 1.61)	10	0.72 (0.39 - 1.33)	34	1.52 (1.07 - 2.10)

IR, incidence rate; PY, person years; CI confidence interval.

Table 4. Time-dependent multivariate model for CIN2+

	Model 1			Model 2		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Immunosuppressive exposure	0.82	0.51 - 1.31	0.420	1.22	0.71 - 2.08	0.453
Age at diagnosis (years)	0.99	0.98 - 1.02	0.789	0.99	0.98 - 1.01	0.527
Current smoking (vs never/past)	2.77	1.77 - 4.32	<0.0001	2.75	1.75 - 4.31	<0.0001
Crohn's Disease (vs. UC/IBDU)	1.09	0.65 - 1.83	0.748	1.06	0.63 - 1.78	0.830
Immunomodulator exposure (cumulative) [a]				1.15	1.08 - 1.24	<0.0001
Biologic exposure (cumulative) [b]				1.15	1.01 - 1.30	0.031
Interaction exposure to immunomodulators and biologics				0.95	0.91-0.99	0.043

HR, hazard ratio; aHR; CI confidence interval. [a]Per each additional year of immunomodulator exposure [b]Per each additional year of biologic exposure
Model 1: all variables were time-fixed variables. Model 2: cumulative exposure to immunomodulators and cumulative exposure to biologic agents were assessed as time-dependent; hazard ratios therefore compared time after exposure with time never exposed.

In patients exposed to IM and BIO, exposure to combination treatment accounted for only 6.7% of exposure time. The overall risk of CIN2+ was similar in patients IS exposed and non-exposed patients, also when adjusting for covariates (HR 0.84 95% CI: 0.52-1.34, **Table 4,** Model 1). When considering cumulative exposure, previous cumulative exposure to IM or BIO was associated with CIN2+. For patients who had not been exposed to any IS, the HR associated with a 1 year longer exposure to IM was 1.15 (95% CI 1.08-1.24 p<0.0001, **Table 4**, Model 2). For patients who had not been exposed to IS, the HR associated with a year longer exposure to BIO was also 1.15 (95% CI 1.01-1.30 p<0.030, Model 2). A statistically significant interaction was observed for patients using both IM and BIO through the course of IBD (**Table 4,** Model 2), indicating that CIN2+ risk was slightly less increased in patients exposed to IM and BIO than in patients exposed to only IM monotherapy or BIO monotherapy (B -0.050 HR 0.95 95% CI 0.91-0.99 p<0.043, **Table 4,** Model 2). The effect of cumulative exposure to IS and CIN2+ risk based on model 2 is illustrated using 3 fictitious patients (**Figure 2**). Sensitivity analyses considering lag time did not meaningfully change the results. The HR of the interaction for patients using both IM and BIO agents remained the same, however the 95% CI was slightly wider so that it now just contained zero (6-month lag time HR 0.95 (CI 0.89-1.05) p 0.068, 12-month lag time HR 0.94 (CI 0.89-1.00) p 0.055, **Supplementary table 2**). Smoking during the course of IBD was strongly associated with the risk of CIN2+ lesions in all models (HR 2.75, 95% CI 1.75-4.31, **Table 4,** Model 2**)**.

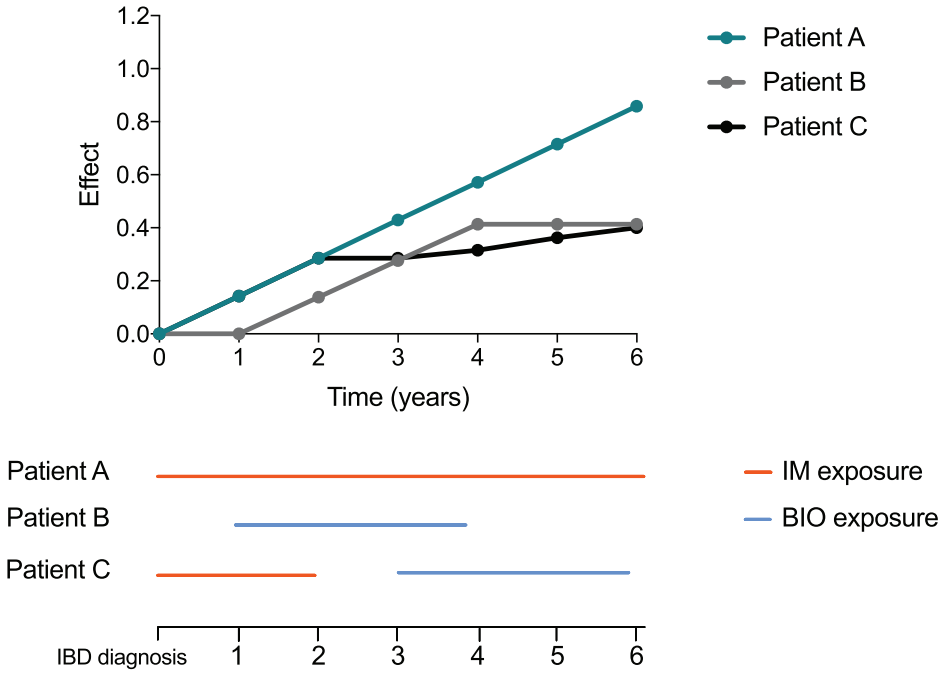

Figure 2. Details of three fictitious patients (A, B, C) illustrating the effect of cumulative exposure to immunosuppressives on CIN2+ risk over time, based on the Cox-regression model. Bottom figure: The red lines represent exposure to immunomodulators (IM) and the blue lines represent exposure to biologic agents (BIO). Patient **A** commenced IM treatment after IBD diagnosis, exposure was continued for the following 6 years. Patient **B** commenced BIO treatment 1 year after IBD diagnosis; exposure was continued for the following 3 years. Patient **C** started IM treatment after IBD diagnosis and was exposed for 2 years and received no treatment with IS for the following year. Three years after IBD diagnosis BIO treatment was initiated and continued for 3 years. The effects of exposure are visualized for each individual patient in the top part of the figure.

DISCUSSION

In this real-world cohort study, the association between exposure to IS (IM and/or BIO) and CIN2+ risk in a large cohort of IBD women with a median follow up of 17.2 years was assessed. Although exposure to IS expressed as dichotomous variables (ever versus never) did not seem to impact CIN2+ development, we demonstrated that each year of exposure to immunomodulators (HR 1.15 per treatment year) or biologics (HR 1.15 per treatment year) was associated with an increased risk of CIN2+ development. Lag-time did not meaningfully change these results. Furthermore, smoking was also a risk factor for CIN2+.

Several other studies have investigated the role of IS use and cervical abnormalities, and so far data have been conflicting. A recent meta-analysis by Allegretti et al reported a significant association between exposure to IS and cervical dysplasia and cancer compared to women in

the general population.[2] However, data should be interpreted with caution since most studies have reported solely on *ever* versus *never* exposure only, disease severity was not taken into account and both high-grade cervical neoplasia an cervical cancer were used as outcome measures separately in the involved studies. Also, a compromised immune system due to illness of IBD itself instead of medication use might have influenced the outcome, as described in studies including women with systemic diseases.[20, 27] Furthermore, most previous studies did not assess the exposure to IS as time-dependent variables, or did not consider lag-time effects of drug exposure on the risk of cervical neoplasia. An increased risk of CIN2+ in IM and anti-TNF monotherapy users has been described in some available studies and mainly in CD patients, however, these data are published variably.[15, 16, 21] Cumulative exposure has only been previously investigated by a Danish study and consistent with our findings, an eight percent increase in incidence rate ratio for CIN2+ risk per redeemed azathioprine prescription in CD patients compared to matched controls was observed.[16] In the same study, there was an increased risk of high-grade cervical dysplasia in CD patients ever exposed to anti-TNF, but an increased risk in cumulative exposure expressed by redeemed prescriptions was not identified.[16]

Literature on the effect of exposure to both IM and BIO on cervical abnormalities is very limited. Marehbian et al evaluated adverse events related to drug exposure in CD patients and reported a HR of 2.34 for cervical dysplasia or HPV in CD patients exposed to both IM and BIO during follow up compared to the general population. Compared to non-exposed CD patients, the HR was 1.52, which was lower than patients only exposed to IM (HR 1.81), but higher than patients only exposed to anti-TNF (HR 1.25).[15] We observed a significant interaction in patients exposed to both IM and BIO, suggesting that that CIN2+ risk was slightly less increased in these patients compared to monotherapy with either IM monotherapy or BIO monotherapy, i.e. not twice increased as expected. However, methodological challenges encountered when studying IBD patients with large heterogeneity in cumulative drug exposure could explain this effect. Only 3.6% of exposure time in the group exposed to both IM and BIO accounted for exposure to combination treatment. This limited exposure time is probably explained by top-down treatment, in which patients are treated with combination treatment with anti-TNF and thiopurines for 6-12 months, and monotherapy with anti-TNF or thiopurines thereafter. Since the exposure time to combination therapy was limited in our cohort, no definitive conclusion can be drawn on its effect on CIN2+ development.

Immunity against hrHPV infection is an important process to eliminate the virus and it involves both cellular innate and adaptive immune responses. The mode of action of thiopurines in inflammatory bowel disease is immunosuppression and mainly involves interference with a cellular inflammation checkpoint Rac1.[28] As a consequence, infections are well-known complications of thiopurine use and mainly the risk of viral infections (EBV, HPV) seems to be increased.[29] Also, thiopurines have a mutagenic potential and several mutations in tumor suppressor genes have been described upon exposure.[30] The malignancy promoting potential of anti-TNF agents is less clear since both anti-tumor effects and pro-tumorogenic effects

have been described. TNF can either stimulate apoptosis and necrosis of tumours via the caspase pathway but also facilitate proliferation of neoplastic cells via the NF-kB pathway.[31] Previous studies have demonstrated that differences in genetic susceptibility and immune responses, including polymorphisms of TNF, are related to the incidence of HPV related lesions and cancer.[32, 33] In this study we show that for each year exposure to IM and BIO (which includes mainly anti-TNF) the risk of CIN2+ increases slightly, supporting that both agents can play a perpetuating role on the cascade from persistent HPV infection towards cervical cancer. Because IS and combination treatment in particular, are often initiated in severe forms of IBD, the association of exposure to IS with an increased risk of CIN2+ may reflect a role of the burden of inflammation rather than of treatment. Although previous studies did provide evidence of an independent role of IBD on the risk of CIN2+, this hypothesis cannot be excluded.

The major strengths of our study are its national scale, large study population, long-term follow-up and data from prospectively maintained registers on IS exposure and cervical neoplasia. The time-dependent approach to exposure classification avoided the introduction of immortal time bias (person-time accumulated between date of diagnosis and date of treatment initiation) that can potentially lead to overestimation of the treatment effect.[34] Also, we were able to correct for important confounders such as smoking and IBD-related factors such as age at diagnosis. Several limitations have to be noted. First, insufficient information on hrHPV status, corticosteroid and oral contraceptive use, pregnancies and sexual behaviour could have prevented identification and control of confounding factors. Second, possible confounding by disease severity and therapy response is a concern as these guide treatment regimens. This IBD cohort comprises patients treated in referral centres reflecting a population with a more complicated disease course with possibly increased drug exposure, and comorbidities that could have influenced CIN2+ risk. Therefore, our results might not be completely generalizable to all IBD patients. Third, heterogeneity of drug regimen and sample size hamper evaluation of the association of combined versus successive exposure to IM and BIO agents and CIN2+ risk. Future large prospective studies will be necessary to assess the risk of cervical neoplasia associated with each individual drug.

Guidelines for cervical screening in IBD vary significantly due to conflicting definitions of the immunocompromised status (as compared to patients suffering from HIV) and conflicting evidence concerning cervical abnormalities.[35] The finding of the association between cumulative exposure and CIN2+ may have implications on the strategies for cervical cancer prevention. Vaccination for HPV should be strongly encouraged in all IBD women up to 26 years of age, ideally in a pre-treatment setting. Normal immunogenic response to HPV vaccination has been reported in patients on immunosuppressive medication, therefore vaccination should not be retained in women on active medical treatment.[36] Also, adherence to national cervical cancer screening programs should be strongly encouraged in all IBD women, especially since testing rates in IBD women remain low.[19, 37] An intensified screening approach could be considered in IBD women exposed to long-term treatment with IS. This

study should further alert physicians on the risks of long-term IS therapy in IBD patients and should encourage them to always consider stopping medication in patients that have long-term remission or quiescent disease. Lastly, although this cohort only included women, IBD is also a risk factor for other HPV-associated neoplasia such as head, neck and anogenital cancers.[38-40] Future studies should investigate the impact of immunosuppressive exposure in other HPV-associated cancers and the efficacy of HPV vaccination in male IBD patients.

In conclusion, long-term exposure to IM and BIO is associated with an increased risk of CIN2+ in IBD women. Physicians should stress the importance of HPV vaccination and cervical cancer screening for all IBD women. These results imply that further investigation is required to assess the benefit of intensified screening IBD women on long-term immunosuppressive treatment.

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SUPPLEMENTARY DATA

Supplementary Table 1. Sensitivity analyses, time-dependent multivariate model for CIN2+ in full cohort

	Model 1			Model 2		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Immunosuppressive exposure	1.57	0.98 – 2.53	0.063	1.15	0.71 - 1.87	0.571
Age at diagnosis (years)	0.99	0.97 - 1.01	0.161	0.99	0.97 - 1.01	0.233
Current smoking (vs never/past)	2.74	1.80 - 4.16	<0.0001	2.70	1.77 - 4.09	<0.0001
Crohn's Disease (vs. UC/IBDU)	0.95	0.59 - 1.53	0.818	1.00	0.62 - 1.61	0.986
IM exposure (cumulative) [a]	1.15	1.08 - 1.23	<0.01	1.07	1.03 - 1.10	<0.0001
BIO exposure (cumulative) [b]	1.14	1.01 - 1.29	0.035	1.12	1.01 - 1.27	0.044
Interaction exposure to IM and BIO	0.95	0.91-0.99	0.045	0.97	0.93-1.00	0.054

[a]Per each additional year of immunomodulator exposure [b]Per each additional year of biologic exposure. Model A: patients with unknown exposure (n=150) were included and coded as never exposure. Model B: patients with unknown exposure (n=150) were included and coded as continuous exposure. Cumulative exposure to immunomodulators and cumulative exposure to biologic agents were assessed as time-dependent; hazard ratios therefore compared time after exposure with time never exposed.

Supplementary Table 2. Sensitivity analyses of time-dependent multivariate model for CIN2+ including lag time

	6-months lag time			12-months lag time		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Immunosuppressive exposure	1.24	0.75 – 2.06	0.405	1.28	0.77 – 2.12	0.349
Age at diagnosis (years)	0.99	0.97 - 1.01	0.435	0.99	0.97 - 1.01	0.421
Current smoking (vs never/past)	2.70	1.72 - 4.23	<0.0001	2.69	1.72 - 4.22	<0.0001
Crohn's Disease (vs. UC/IBDU)	0.93	0.58 - 1.55	0.781	0.93	0.56 - 1.55	0.775
IM exposure (cumulative) [a]	1.15	1.07 - 1.23	<0.01	1.16	1.08 - 1.25	<0.0001
BIO exposure (cumulative) [b]	1.13	1.00 - 1.29	0.050	1.15	1.00 - 1.32	0.047
Interaction exposure to IM and BIO	0.95	0.89-1.05	0.068	0.94	0.89-1.00	0.055

[a]Per each additional year of immunomodulator exposure [b]Per each additional year of biologic exposure. Drug exposure was assessed as time-dependent variables and exposure time was lagged for 6 months and 12 months respectively.

Supplementary Table 3. Characteristics drug exposure IBD cohort

	Total cohort n=1981
Immunosuppressive exposure	1305 (62.4)
Immunomodulators	1155 (58.3)
azathioprine	928 (46.8)
mercaptopurine	331 (16.7)
thioguanine	77 (3.9)
methotrexate	276 (13.9)
calcineurin inhibitors	60 (3.0)
other/unspecified	42 (2.1)
Biologic agents	795 (40.1)
infliximab	579 (29.2)
adalimumab	433 (21.9)
certolizumab	17 (0.9)
golimumab	11 (0.6)
vedolizumab	39 (2.0)
ustekinumab	13 (0.7)
other/unspecified	14 (0.7)

Values are expressed as total number (n) and percentages (%)

Supplementary Table 4. Characteristics cohort cumulative drug exposure

	Total cohort n=1831
Immunosuppressive exposure	1145 (62.5)
Unexposed	686 (37.5)
Immunomodulators	1002 (54.7)
monotherapy	449 (24.5)
Biologic agents	696 (38.0)
monotherapy	143 (7.8)
Immunomodulator & Biologic agents	553 (30.2)
Sequential	225 (12.3)
Combination	328 (17.9)
Immunomodulator first	336 (18.6)
Biologic agent first	148 (8.1)
Initiated as combotherapy	71 (3.9)

Values are expressed as total number (n) and percentages (%)

PART III

LOCAL TREATMENT

CHAPTER 9

NO SUPERIORITY OF TACROLIMUS SUPPOSITORIES VS BECLOMETHASONE SUPPOSITORIES IN A RANDOMIZED TRIAL OF PATIENTS WITH REFRACTORY ULCERATIVE PROCTITIS

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ABSTRACT

Background: 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.

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 but may also reduce the risk of progression of disease extent.[2] 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%, maintenance of remission occurs in only 50% of patients.[6] 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.

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 (**Figure 1**). In total, 43 patients received tacrolimus and 42 received beclomethasone (**Table 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 1**. The study ended after the last follow-up visit from the last patient, in December 2017.

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).

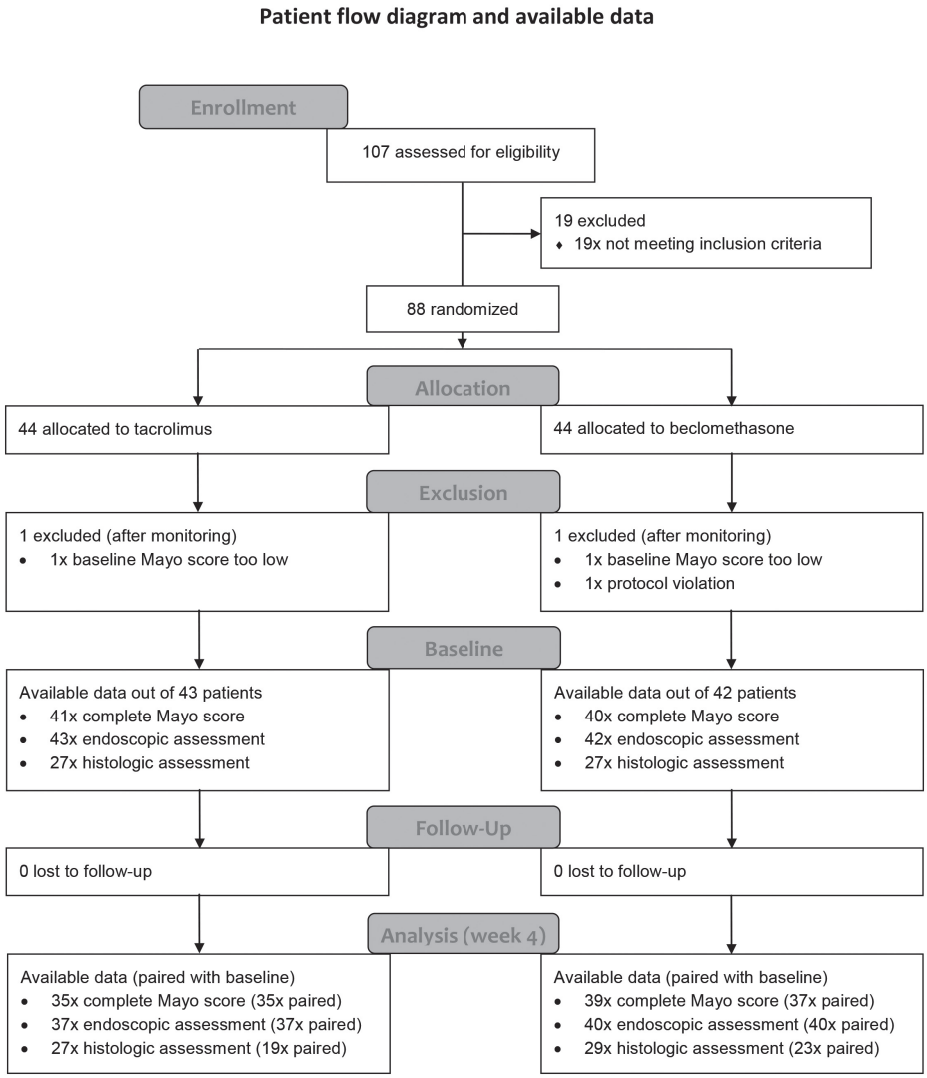

Figure 1. CONSORT flow diagram of screened and included patients, and available study data per time-point.

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 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 2B**).

Table 1. Baseline characteristics of included patients

	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)

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 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 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 ($p = 0.299$).

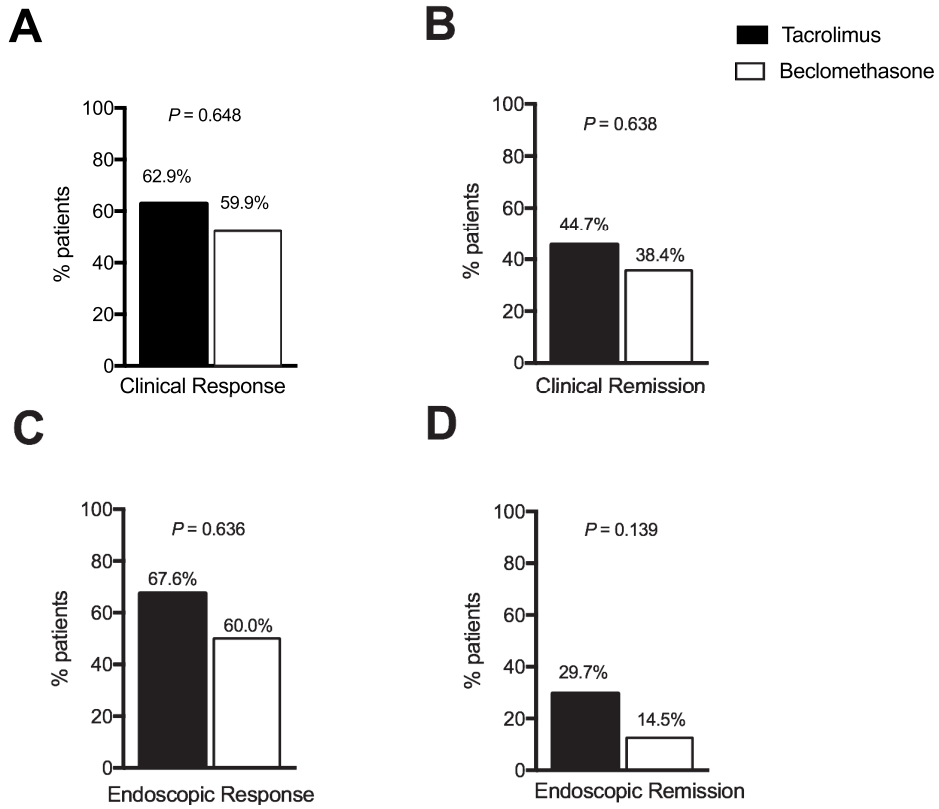

Figure 2. Main study results. All panels show tacrolimus as white and beclomethasone as gray. Panel A shows the proportion of patients with a clinical response, panel B shows the proportion of patients with clinical remission. In panel C the proportion of patients with endoscopic response is shown and panel D shows the proportion of patients with endoscopic remission.

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 remainders 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 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 2. Overview of adverse events

	Tacrolimus	Beclomethasone	Total
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, certain differences in the reported outcomes warrant consideration.[13] 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 criterion 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 2 mg 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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SUPPLEMENTARY DATA

Supplementary data 1. 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.

PART IV

DISCUSSION

CHAPTER 10

SUMMARY, GENERAL DISCUSSION AND FUTURE PERSPECTIVES

SUMMARY

This thesis aimed at providing more insight into several safety aspects, efficacy and opportunities with current immunosuppressive treatment in inflammatory bowel disease (IBD). First we explored risk factors for therapy withdrawal due to ineffectiveness or adverse events. Second, we focussed on the risk of cervical dysplasia and cervical cancer in IBD and the role of immunosuppressive therapy in these extra-intestinal complications. Finally, we explored opportunities in treatment and monitoring of IBD patients. In this chapter, we summarize and discuss these aspects and provide possible directions for future research.

GENERAL DISCUSSION

Risk factors for therapy failure

Difficulties in thiopurine treatment

Although thiopurine treatment for IBD has been introduced several decades ago, the balance between optimization of treatment effect and avoidance of toxicity remains an important challenge in clinical practice.[1-3] Strategies to monitor this balance include genetic testing of polymorphisms associated with risk of toxicity (e.g. TPMT, NUDT15), therapeutic drug monitoring (TDM) of thiopurine metabolites 6-thioguanine nucleotides (6-TGN) and 6-methylmercaptopurine ribonucleotides (6-MMPR), and laboratory monitoring of toxicity.

In **Chapter 2** we have studied treatment failure within a cohort of 49 IBD patients with a routinely established skewed metabolism, i.e. a drug metabolism variation that results in excessive amounts of cytotoxic 6-MMPR at the expense of immunosuppressive 6-TGN levels that usually remain below the therapeutic range. In this cohort, 84% of patients discontinued thiopurine therapy in the first 3 months of treatment, mainly for adverse events (55%) and nonresponse (12%). This proportion of early failure of thiopurine therapy was substantially higher than the 15-35% reported in literature on early failure.[4,5] We concluded that a skewed metabolism of thiopurines is a major risk factor for future thiopurine failure. TDM is usually applied as a reactive strategy when a patient does not adequately respond to treatment or experiences intolerable adverse events and therefore a skewed metabolism is generally detected upon signs of toxicity or treatment failure.[6-8] Our observations imply that routine thiopurine metabolite measurements early after the start of thiopurines may be considered to identify patients with an aberrant-skewed metabolism at an early stage, possibly benefitting from therapy adjustments, to prevent thiopurine therapy failure. This is supported by the observation by Wong et al that assessment of 6-TGN and 6-MMPR metabolites as early as 1 week after thiopurine initiation (i.e. before steady-state) was correlated with the development of leukopenia and hepatotoxicity.[9,10]

Since up to 37% of IBD patients harbour thiopurine metabolism variations, early (pre-emptive) TDM-guided thiopurine therapy deserves further study in a prospective cohort

study, with a detailed analysis of (long-term) efficacy, toxicity and cost-effectiveness. For the development of this monitor strategy the importance of genetic polymorphisms in thiopurine S-methyltransferase (TPMT) and NUDT15 associated with therapy withdrawal because of myelotoxicity need to be taken into account. Genetic variants in NUDT15 is particularly present in patients of Asian descent and to a much lesser extent in patients of non-Asian descent.[11,12] However, it should be noted that both of these genetic variations only explain a proportion of myelotoxicity cases and myelotoxicity is not the only reason for therapy withdrawal. In TDM the outcome of genetic variations and inadequate dosing is detected and it is uncertain if extensive pre-treatment pharmacogenetic screening will replace TDM in the future. Based on current literature and the findings in our study, a monitor strategy combining pre-treatment TPMT and NUDT15 testing, and early TDM at week 8 seems advocated to prevent patients from discontinuing thiopurine treatment.

In **Chapter 3** we demonstrated that patients with a skewed thiopurine metabolism are at increased risk of myelotoxicity. Although myelotoxicity, in particular leukopenia, during thiopurine treatment is most commonly attributed to (extremely) high 6-TGN levels, we have shown in this study of 24 patients that myelotoxicity also occurs because of (extremely) high 6-MMPR concentrations in patients with a skewed thiopurine metabolism. Leukopenia improved in 17% and resolved in 83% of patients within 4 weeks of adjusting treatment (i.e. discontinuation, LDTA or switch to thioguanine (TG)). These data confirm the value of TDM after detection of laboratory toxicity, c.q. leukopenia. Myelotoxicity, especially leukopenia, is usually attributed to high bioavailability of 6-TGN levels as a result of overdosing or genetic variation resulting in low TPMT enzyme activity.[13,14] Pre-treatment genetic testing of TPMT is performed to identify patients with absent or low TPMT enzyme activity that are prone to development of myelotoxicity and would benefit from reduced thiopurine dose or treatment with a different drug. Because 6-MMPR formation is mainly driven by TPMT activity (i.e. normal to high activity), pre-treatment genetic testing would not have prevented myelotoxicity in these patients. These study findings confirm previous publications that TDM is particularly useful in the management of patients with inadequate response to thiopurines or adverse events.[15] In addition, reactive TDM has been shown to optimize treatment response and is cost-effective compared with standard weight-based dosing.[16,17]

Optimizing thiopurine treatment

Several strategies have been proposed to optimize thiopurine treatment in patients with inadequate response or adverse events (AEs). Low dose thiopurine combined with allopurinol (LDTA) therapy is a beneficial optimization strategy in patients with a skewed thiopurine metabolism but data on continued maintenance LDTA treatment and incidence of renewed laboratory toxicity are scarce.[18 19-21] In **Chapter 4** we demonstrated that maintenance LDTA is a safe and beneficial optimization strategy in a cohort of 221 IBD patients with treatment continued in 51% of patients beyond 5-years. Treatment withdrawal was most commonly the result of ineffectiveness of LDTA rather than LDTA-attributed laboratory toxicity. Importantly, we showed that LDTA is most advantageous in patients failing thiopurine monotherapy due

to AEs with high rates of continued treatment and rapid recovery of thiopurine monotherapy associated hepatotoxicity and also myelotoxicity. In addition, none of these patients stopped treatment for LDTA-induced laboratory toxicity. Given the favourable effects of allopurinol on thiopurine metabolism and long-term therapeutic benefit and safety, one could argue that LDTA should not be restricted to patients with a skewed metabolism. In the presence of pre-treatment genetic screening and/or pre-emptive TDM, LDTA could possibly be considered as a first-line therapy. These predictions await confirmation by the on-going multicentre randomised controlled DECIDER trial (ACTRN12613001347752), evaluating response and tolerability of thiopurine monotherapy compared to LDTA in thiopurine naïve patients.

The advantage of tioguanine is that only one pill has to be administered which may improve treatment adherence and avoids the toxicity of a second drug such as allopurinol. In contrast, therapy with LDTA is supported by more clinical evidence that may guide the interpretation of the levels of metabolites associated with effectiveness and safetye advantage of tioguanine is that only one pill has to be administered which may improve treatment adherence and avoids the toxicity of a second drug such as allopurinol. In contrast, therapy with LDTA is supported by more clinical evidence that may guide the interpretation of the levels of metabolites associated with effectiveness and safety.

Improving thiopurine monitoring

As hepatotoxicity and myelotoxicity often result in thiopurine withdrawal, frequent monitoring of laboratory parameters including a full blood count (FBC) and serum liver enzyme tests (LTs) is performed to guide possible therapy modification.[22-25] The risk of laboratory toxicity is highest in the first months of treatment, but after this induction period it is unclear if the potential benefit of laboratory monitoring at 2-3 month in maintenance therapy outweighs the burden for patients and associated direct and indirect healthcare costs.[26] In **Chapter 5** we assessed incidence rates and clinical consequences of myelotoxicity and hepatotoxicity within current laboratory regimen in thiopurine maintenance therapy after at least 1 year of treatment in a cohort of 1132 IBD patients. Although mild myelotoxicity and hepatotoxicity were present (5% and 7% per treatment year), severe laboratory toxicity was uncommon after 1 year of thiopurine monotherapy (0.1% and 0.2%). Our results demonstrated that laboratory monitoring rarely leads to therapy adjustments or additional diagnostic procedures such as additional laboratory assessments. The most relevant observation was that severe toxicity-related complications, such as infection and hospitalisation, were rare (1% in the total cohort) and not prevented by (stringent) monitoring, as preceding laboratory assessments were unremarkable in the majority of cases. Since the clinical impact of detecting laboratory toxicity in maintenance therapy was low, and complications rare, routine monitoring is of limited benefit. Laboratory monitoring practices in our large real-life cohort were more liberal than recommended in the ECCO guideline; at 4-month intervals (our study) versus at 2-3 month intervals (ECCO guideline).[27] Therefore, we conclude that the recommended monitoring frequency may be reduced to less than every 4 months. The rate of thiopurine-induced myelotoxicity has been reduced after the introduction of TPMT genotype testing.[26,28]

In addition, Broekman et al showed that patients with non-TPMT mediated thiopurine-induced leukopenia had no increased infection risk compared to patients without leukopenia.[29] These results also support that monitoring frequency in maintenance thiopurine treatment could be reduced.

We speculate that a monitoring regimen at 6-month intervals is sufficient in patients after 1 year of thiopurine treatment and will decrease patient burden and healthcare costs. To confirm these speculations, prospective evaluation in an impractically large study population with several years of follow-up would be required. In addition, detection of laboratory toxicity is expected to influence both treatment and monitoring and this will not be avoided by a prospective design. Therefore, a prospective cohort study will probably not provide the required data to test this hypothesis. Our results apply to patients on thiopurine monotherapy and it would be interesting to study the clinical relevance of laboratory toxicity in patients on combination therapy with biologic agents.

Difficulties in adalimumab treatment

Biologic agents are increasingly used to treat IBD patients, but drug efficacy and tolerability are often different in real-life clinical practice than reported in randomised controlled trials performed under strict conditions in a selected patient population. To allow optimal drug use in a personalized fashion, factors that predict treatment failure, safety and tolerability need to be studied in clinical cohorts. In **Chapter 6** we described a prospective real-life cohort study of 188 CD patients treated with adalimumab and assessed efficacy and tolerability. In particular we studied possible sex differences in the outcome to adalimumab treatment and we noticed that that female sex negatively affects treatment success of adalimumab. First, female patients were less likely to achieve or maintain clinical response than male patients (31% vs 48%). Second, female patients reported more side effects such as skin reactions and infections, but more importantly stopped ADA treatment more often than males due to side effects. This suggests that a personalized approach to female patients starting ADA could positively influence treatment success. In other fields of medicine it has been shown that the sex of the patient can have profound influences on drug metabolism and efficacy, also in biological treatment.[30,31] Although the exact mechanism is unknown, our results could be explained by sex specific physiological differences in drug metabolism, body composition or sex hormones, [32-41] Apart from physiological explanations, perception of adverse events and health care seeking behaviour also differs between males and females. In a recent systematic review performed by Lie et al, no evidence was found for sex differences in efficacy of biological therapies using objectively measured endoscopic disease outcomes.[42] This finding strongly suggests that the greater proportion of side effects and subsequent therapy withdrawal is the major cause for a decreased success rate of ADA in females. Providing tailored information and education concerning the possible side effects may result in different patient expectations, which could subsequently reduce the high drop out rate due to side effects, as observed in our study.

Cervical neoplasia in IBD

Cervical neoplasia in IBD

Although several studies suggest that IBD women are at increased risk of cervical neoplasia caused by human papilloma virus (HPV), the role of disease characteristics and immunosuppression in the pathogenesis is unclear.[43-47] Due to conflicting evidence and lack of longitudinal follow-up in the majority of studies it is unclear whether cervical screening in IBD needs to be intensified. In **Chapter 7**, we studied the risk of high-grade cervical dysplasia and cancer (CIN2+) in a large cohort of IBD women compared to a matched cohort from the Dutch general female population with longitudinal follow up. We found that CIN2+ detection rate was higher in the IBD cohort than in the matched cohort, even after correcting for their more frequent screening behaviour (standardised detection rate 1.27). This risk was highest in women within the age group of 35 to 39 years (standardised detection rate 1.80). In line with previous findings we found that smoking was a strong risk factor for CIN2+, but CIN2+ risk associated with smoking was higher in IBD than in the general population.[46,48-51] Therefore, one may suggest a combined effect of IBD and exposure to cigarette smoke on CIN2+ risk which provides a further strong motivation to encourage patients to stop smoking. Exposure to immunomodulators and biologics, studied as trichotomous variables (never, <1 year, > 1 year), was not associated with CIN2+ development. Importantly, we show that the risk of progression from normal cervical smear towards CIN2+ was increased in IBD women, and IBD women were also at increased risk for persistent or recurrent CIN than women in the general population. Reduced high-risk HPV (hrHPV) clearance due to impaired detection of oncogenic signals (immunosurveillance) caused by chronic systemic inflammation could explain these findings.[52-55] This is supported by the fact that we did not establish a difference in transient low-grade CIN1 between both groups. Therefore, vaccination for HPV and adherence to cervical cancer screening programs should be strongly encouraged in all IBD women, regardless of immunosuppressant use. This is supported by the fact that normal immunogenic response to HPV vaccination has been reported in patients on immunosuppressive medication.[56]

In **Chapter 8** we further explored the effect of exposure to immunosuppressive drugs (immunomodulators and/or biologics) and CIN 2+ development in the IBD cohort. Although exposure to immunosuppressive drugs expressed as dichotomous variables (ever/never) did not seem to impact CIN2+ development, we demonstrated that each year of exposure to immunomodulators (HR 1.15 per treatment year) or biologics (HR 1.15 per treatment year) was associated with an increased risk of CIN2+ development. Lag-time of 6 and 12 months taking latency time of drug exposure and CIN2+ development into account, did not meaningfully change these results. The influence of immunosuppressive medication on CIN2+ development could again be explained by a decreased clearance of hrHPV infections caused by an (iatrogenic) immune-compromised state. Physicians should stress the importance of HPV vaccination and cervical cancer screening for all IBD women. These results imply that further investigation is required to assess the benefit of intensified screening IBD women on long-term immunosuppressive treatment.

Local treatment of ulcerative proctitis

Topical therapies are an attractive treatment option in ulcerative proctitis because of their local activity and minimal systemic toxicity. Unfortunately, the arsenal of topical therapies is small and patients with 5-ASA refractory proctitis often require step up treatment to (local) corticosteroids, immunomodulators or biological agents that might be associated with adverse events and higher costs. Previous studies reported promising results of tacrolimus as a treatment for proctitis compared to placebo.[57,58] **Chapter 9** describes a multicentre double-blinded randomized controlled trial aimed to evaluate tacrolimus suppositories 2mg OD compared to beclomethasone 3mg OD suppositories for 4 weeks in patients with 5-ASA refractory UP. We demonstrated that tacrolimus and beclomethasone suppositories display comparable clinical and endoscopic response. Both drugs managed to induce clinical response (60%) and endoscopic response (60%) in the majority of patients. Furthermore, both treatments resulted in improvements in histological inflammation and quality of life. We did not observe significant differences in adverse events rates between tacrolimus and beclomethasone. Our findings imply that both tacrolimus and beclomethasone suppositories are viable treatment options for 5-ASA refractory ulcerative proctitis. Adequate treatment of UP is not only important for symptom control and quality of life, but also to reduce disease progression.[59-62] Topical treatment with tacrolimus or beclomethasone should be considered in 5-ASA refractory UP prior to step-up to systemic treatment with thiopurines or biologic agents. It would be interesting to study real-life effectiveness and tolerability of continued (maintenance) treatment with tacrolimus in UP in the future.[63]

FUTURE PERSPECTIVES

Selecting the right drug for the right patient in the expanding maze of IBD treatment is a true challenge. Appropriate treatment of IBD is essential to avoid long-term disease complications. Despite significant efforts, a cure for IBD has not been identified. Although there is a definite need for novel and improved treatment options to maximise remission rates and quality of life, there is a lot of room for improvement with the currently available treatments.

Apart from effectively targeting the perpetuating inflammatory response in IBD with medication, efforts should be made to further optimize monitoring of treatment response and adverse events and adequate optimization strategies to prevent treatment failure and improve clinical outcome. Early (pre-emptive) TDM-guided thiopurine therapy seems promising to prevent thiopurine failure and future prospective studies should focus on (long-term) efficacy, toxicity and cost-effectiveness of this strategy, possibly combined with pre-treatment genetic testing. Ideally, genotyping of all patients is performed prior to thiopurine exposure to reveal genetic variations that influence thiopurine drug metabolism to ultimately prevent patients from being exposed to drugs that are not effective or harmful due to severe toxicity and would therefore benefit from alternative therapies. However, as TDM essentially comprises the outcome of all genetic variations and inadequate dosing, it is questionable if pre-treatment

pharmacogenetic testing will eventually alleviate the burden of routine monitoring for toxicity in the future.

Optimization strategies with LDTA and TG show similar benefit in patients failing conventional thiopurines, but because of their favourable properties in thiopurine metabolism, the efficacy of first-line treatment with LDTA of TG should be further studied, preferably in a head-to-head trial.

Another aspect to explore is thiopurine-analogues that specifically target the Rac1 signalling pathway to increase T-cell apopotosis and bypassing enzymatic pathways associated with toxicity. Optimised immunosuppressive agents might offer relevant therapy implications if confirmed in clinical studies. Although the vast majority of new immunosuppressive drugs are systemic therapies, rectal therapies that clearly offer a number of advantages remain underdeveloped. To allow for personalized medicine in patients with distal forms of IBD, (new) local therapies should be explored.

In (severely) active IBD the risks of medical treatment generally outweigh possible disease-related complications, but the risks of long-term immunosuppressive treatment need to be noted. Since cervical neoplasia is increased in IBD women, future studies should aim at exploring persistence of hrHPV and the role in cervical carcinogenesis as this may lead to strategies of (temporal) reduction of immunosuppressant use to clear the virus and halt or even reverse cervical carcinogenesis, before passing a point of no return. Oncogenic HPV-infections are also associated with head, neck and anogenital cancers besides cervical cancer. Current estimates indicate that 5.2% of all cancers are HPV associated and the risk of infection is equal among men and women, therefore future studies should also investigate the impact of immunosuppressive exposure in other HPV-associated cancers and the efficacy of HPV vaccination in male IBD patients.

An important step in the translation of our results in to clinical practice would be the development of a prediction tool allowing for personalized medicine. The inherent complexity of IBD introduces a large number of confounding factors that hamper development of a personalized treatment strategy. It is unlikely that RCTs will yield the evidence for such a strategy as these studies use strict in- and exclusion patient criteria and therefore do not represent clinical practice. Consistent longitudinal collection of clinical data and biomaterials in nationwide databases, such as the Dutch IBD biobank (PSI) described in this thesis, could allow for development of a therapeutic strategy for each individual IBD patient. Future research should explore further development of personalized treatment strategies, preferably in the setting of international collaboration. A new high-potential field of research to analyse complex clinical data combined with data from biomaterials is system dynamics analysis, which allows for real-time individualized prediction of disease and treatment outcomes.[64]

To conclude, IBD is a complex disease with large inter-individual variance in disease behaviour and treatment response. Overall, this thesis adds to the current data regarding safety aspects of current medical immunosuppressive treatment in IBD and provides several opportunities to improve IBD care. Combining these findings together with clinical data and biomaterials could guide development of more tailored treatment strategies will hopefully become reality in the near future.

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APPENDICES

APPENDIX

NEDERLANDSE SAMENVATTING

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Inleiding

Inflammatoire darmziekten (IBD) zijn chronische inflammatoire aandoeningen die voornamelijk het maagdarmkanaal aantasten. IBD wordt gekenmerkt door terugkerende chronische ontsteking met periodes van actieve ziekte afgewisseld met perioden waarin de ziekte rustig is. De belangrijkste ziektebeelden zijn de ziekte van Crohn (CD), colitis ulcerosa (UC) en IBD-niet-geclassificeerd (IBDU). Deze ziekten hebben deels overlappende kenmerken, maar onderscheiden zich in verschil in de locatie en het gedrag van de ontsteking. IBD is een complexe, multifactoriële ziekte en ondanks aanzienlijke inspanningen is er nog geen manier gevonden om de ziekte te genezen. Aangezien IBD een chronische ziekte is en gepaard gaat met invaliderende symptomen is langdurige behandeling met ontstekingsremmende geneesmiddelen in de meeste patiënten noodzakelijk, soms zelfs levenslang. Het doel van deze behandeling is om opvlamming te behandelen zodat de ziekte tot rust kan komen (remissie) en een nieuwe opvlamming te voorkomen. Wanneer de ziekte niet voldoende wordt behandeld kan dit leiden tot onomkeerbare schade aan het maag-darm stelsel, verminderde kwaliteit van leven en werkproductiviteit, en hoge zorgkosten. Anderzijds hebben medicijnen ook bijwerkingen en kunnen behandelings-gerelateerde complicaties zoals infecties of kanker voorkomen. Het is een uitdaging om de juiste behandeling voor de juiste patiënt te selecteren in de klinische praktijk, waarbij een balans gezocht moet worden tussen de effectiviteit van het medicijn, de bijwerkingen en de kosten.

Met dit proefschrift hebben we getracht om meer inzicht te geven in verschillende veiligheidsaspecten, effectiviteit en mogelijkheden binnen huidige behandelingen voor IBD. In deel I hebben we risicofactoren onderzocht voor het staken van behandeling vanwege ineffectiviteit of bijwerkingen. Ten tweede hebben we ons gericht op het risico van baarmoederhalskanker en voorloperstadia bij IBD en de rol van immunosuppressieve therapie. Ten slotte hebben we mogelijkheden van lokale therapie onderzocht bij patiënten met colitis ulcerosa waarbij het laatste gedeelte van de dikke darm is aangetast. In dit hoofdstuk vatten we deze aspecten samen en bespreken we suggesties voor toekomstig onderzoek.

Risicofactoren voor therapiefalen

Uitdagingen in thiopurine therapie

Hoewel thiopurines al decennia lang worden toegepast in de behandeling van IBD, blijft het in de klinische praktijk een uitdaging om de balans te vinden tussen enerzijds het optimaliseren van het behandelefect en anderzijds het vermijden van toxiciteit. Strategieën om deze balans te bewaken omvatten genetische testen op polymorfismen die verband houden met het risico op toxiciteit (bijv. TPMT, NUDT15), *therapeutic drug monitoring* (TDM) van thiopurinemetabolieten 6-thioguaninenucleotiden (6-TGN, werkzame metabolieten) en 6-methylmercaptopurine-ribonucleotiden (6-MMPR, toxische metabolieten), en monitoren van toxiciteit in het bloedonderzoek (lab monitoring).

In **Hoofdstuk 2** is de voorspellende waarde van een afwijkend 'skewed' thiopurine metabolisme bestudeerd, vastgesteld bij routinematige metingen in bloedonderzoek, in een cohort van 49 IBD patiënten. Een skewed thiopurine metabolisme is een variatie die resulteert in overmatige hoeveelheden toxische 6-MMPR ten koste van immuunsuppressieve 6-TGN-niveaus die vaak geen therapeutische waarde bereiken. In dit cohort stopte 84% van de patiënten met thiopurine therapie binnen de eerste 3 maanden van de behandeling, voornamelijk vanwege bijwerkingen (55%) en uitblijvende respons (12%). Dit percentage van vroegtijdig falen van thiopurinetherapie was substantieel hoger dan 15-35% zoals in de literatuur gerapporteerd. We concludeerden dat een skewed metabolisme een belangrijke risicofactor is voor toekomstig falen van thiopurines. TDM wordt gewoonlijk toegepast als een reactieve strategie wanneer een patiënt niet adequaat reageert op de behandeling of ondraaglijke bijwerkingen ervaart. Hierdoor wordt een skewed metabolisme over het algemeen pas gedetecteerd bij tekenen van toxiciteit of falen van de behandeling. Deze resultaten impliceren dat overwogen kan worden om thiopurinemetabolieten routinematig te bepalen, vroegtijdig na het starten van de thiopurinebehandeling om zo patiënten met een skewed metabolisme in een vroeg stadium te identificeren. Deze patiënten hebben mogelijk baat bij aanpassing van de thiopurine therapie, waardoor voorkomen kan worden dat de behandeling faalt. Dit advies wordt ondersteund door de resultaten van een studie van Wong et al, waarin 6-TGN- en 6-MMPR-metabolieten een week na aanvang van de thiopurine behandeling gecorreleerd waren met de ontwikkeling van leukopenie en levertoxiciteit. Aangezien tot 37% van de IBD-patiënten variaties in het thiopurinemetabolisme herbergen, is het zinvol om vroege (preventieve) TDM-geleide thiopurine therapie nader te onderzoeken in een prospectieve cohortstudie, met een gedetailleerde analyse naar (lange termijn) werkzaamheid, toxiciteit en kosteneffectiviteit. Voor de ontwikkeling van deze nieuwe monitorstrategie moet rekening worden gehouden met het belang van genetische polymorfismen in thiopurine S-methyltransferase (TPMT) en NUDT15 die vanwege beenmergtoxiciteit geassocieerd met het stoppen van therapie. Genetische varianten in NUDT15 zijn vooral aanwezig bij patiënten van Aziatische afkomst en in veel mindere mate bij patiënten van niet-Aziatische afkomst. Het feit dat deze twee genetische variaties slechts een deel van de beenmergtoxiciteit verklaren en toxiciteit van het beenmerg niet de enige reden is van therapie falen verdiend de aandacht. Bij TDM komen in principe de uitkomsten van zowel genetische variaties en inadequate dosering aan het licht en het is nog niet duidelijk of uitgebreide genetische screening voorafgaand aan de behandeling, TDM in de toekomst volledig zal vervangen. Op basis van de huidige literatuur en de bevindingen in onze studie lijkt screening naar TPMT- en NUDT15 polymorfismen voorafgaand aan de behandeling combineert met vroege TDM 8 weken na aanvang van de behandeling de aanbevolen monitorstrategie om te voorkomen dat patiënten de thiopurine behandeling staken.

In **Hoofdstuk 3** hebben we aangetoond dat patiënten met een skewed thiopurine metabolisme een verhoogd risico hebben op beenmergtoxiciteit. Hoewel beenmergtoxiciteit, in het bijzonder leukopenie, tijdens thiopurine therapie meestal wordt toegeschreven aan (extreem) hoge 6-TGN-spiegels, hebben we in deze studie onder 24 patiënten met een skewed

metabolisme aangetoond dat beenmergtoxiciteit ook optreedt in aanwezigheid van (extreem) hoge 6-MMPR-concentraties. Leukopenie verbeterde bij 17% en verdween bij 83% van de patiënten binnen 4 weken na aanpassing van de behandeling (d.w.z. staken van thiopurine, lage dosis thiopurine in combinatie met allopurinol (LDTA) of switch naar thioguanine therapie (TG)). Deze resultaten bevestigen de waarde van TDM ook na detectie van laboratoriumtoxiciteit, c.q. leukopenie. Beenmergtoxiciteit, met name leukopenie, wordt gewoonlijk toegeschreven aan een hoge biologische beschikbaarheid van 6-TGN-metabolieten als gevolg van overdosering of genetische variatie die leidt tot verminderde of afwezige enzym activiteit van thiopurine-S-methyltransferase (TPMT). Genetische screening naar TPMT mutaties wordt voorafgaand aan therapie verricht om patiënten te identificeren die door lage of afwezige enzymactiviteit vatbaar zijn voor de ontwikkeling van beenmergtoxiciteit en baat zouden kunnen hebben bij het verlagen van de dosis of behandeling met een ander geneesmiddel. Omdat de vorming van 6-MMPR voornamelijk wordt veroorzaakt door TPMT-activiteit (d.w.z. normale tot hoge activiteit), zou beenmergtoxicteit bij deze patienten niet voorkomen zijn door een genetische test voorafgaand aan de behandeling. Deze onderzoeksresultaten bevestigen dat TDM bijzonder nuttig is bij de behandeling van patiënten met onvoldoende respons op thiopurines of bijwerkingen. Bovendien is aangetoond dat reactieve TDM de respons op de behandeling optimaliseert en kosteneffectief is vergeleken met standaard dosering gebaseerd op het gewicht.

Optimaliseren van thiopurinetherapie

Er zijn verschillende strategieën om de thiopurinebehandeling te optimaliseren bij patiënten met onvoldoende respons of bijwerkingen (AEs). Behandeling met een lage dosis thiopurine gecombineerd met allopurinol (LDTA) is een gunstige optimalisatiestrategie bij patiënten met een skewed thiopurine metabolisme, maar gegevens over voortgezette onderhoudsbehandeling met LDTA en het voorkomen van hernieuwde laboratoriumtoxiciteit zijn schaars**.** In **hoofdstuk 4** hebben we aangetoond dat onderhoudsbehandeling met LDTA een veilige en gunstige optimalisatiestrategie in een cohort van 221 IBD-patiënten, waarbij de behandeling bij 51% van de patiënten na 5 jaar nog werd gebruikt. Daarbij zagen we dat de het staken van de behandeling meestal het gevolg was van onvoldoende effectiviteit van LDTA en niet door laboratoriumtoxiciteit toegeschreven aan LDTA. Belangrijk is dat we hebben aangetoond dat patiënten die vanwege bijwerkingen falen op thiopurine monotherapie het meeste baat hebben bij LDTA. In die groep wordt de behandeling grotendeels voortgezet en treedt er snel herstel op van de lever- en beenmergtoxiciteit geassocieerd met eerdere thiopurine monotherapie. Bovendien stopte geen van deze patiënten de behandeling vanwege LDTA-geassocieerde laboratoriumtoxiciteit. Gezien de gunstige effecten van allopurinol op het thiopurine metabolisme, therapeutische voordeel en de veiligheid op lange termijn, zou men kunnen stellen dat behandeling met LDTA niet beperkt zou moeten worden tot patiënten met een skewed metabolisme. In aanwezigheid van genetische screening voorafgaand aan de behandeling en / of preventieve TDM, zou LDTA mogelijk ook als eerstelijnsbehandeling kunnen worden beschouwd. Echter, onze voorspellingen zijn nog in afwachting van klinische bevestiging in de lopende multicenter gerandomiseerde gecontroleerde DECIDER-studie

(ACTRN12613001347752), waarin de respons en tolerantie van thiopurine monotherapie wordt vergeleken met LDTA in thiopurine-naïeve patiënten.

Verbeteren van thiopurine monitoring

Aangezien beenmergtoxiciteit en levertoxiciteit vaak leiden tot het staken van thiopurine therapie, wordt laboratoriumparameters, waaronder een volledig bloedbeeld en serum leverenzymtesten, frequent gecontroleerd om zo richting te geven aan eventuele therapiewijzigingen. Het risico op deze laboratoriumtoxiciteit is het grootste tijdens de eerste maanden van behandeling (inductieperiode), maar het is onduidelijk of het potentiële voordeel van het bepalen van deze laboratoriumparameters elke 2-3 maanden tijdens onderhoudsbehandeling opweegt tegen de belasting voor patiënten en de bijbehorende directe en indirecte zorgkosten. **Hoofdstuk 5** beschrijft de incidentie en klinische gevolgen van beenmergtoxiciteit en levertoxiciteit binnen het huidige regime van laboratoriummonitoring tijdens thiopurine onderhoudstherapie ten minste 1 jaar na het starten van de behandeling in een cohort van 1132 IBD patiënten. Hoewel we milde beenmergtoxiciteit en levertoxiciteit zagen (5% en 7% resp. per behandeljaar), werd ernstige laboratoriumtoxiciteit maar zelden gezien (0,1%). Onze resultaten toonden aan dat laboratoriummonitoring zelden leidt tot aanpassingen in therapie of aanvullende diagnostische procedures zoals extra laboratoriumbeoordelingen. De meest relevante observatie was dat ernstige toxiciteit-gerelateerde complicaties, zoals infecties en ziekenhuisopname, zeldzaam waren (1% van het totale cohort) en dat (strikte) monitoring deze complicaties niet kon voorkomen aangezien voorafgaande laboratoriumbeoordelingen in de meeste gevallen niet afwijkend waren. Aangezien de klinische impact van het detecteren van laboratoriumtoxiciteit tijdens onderhoudsbehandeling laag was en complicaties zeldzaam waren, is het voordeel van routinematige monitoring beperkt. Opvallend genoeg zagen we dat de frequentie van laboratoriummonitoring in ons grote real-life cohort liberaler was dan werd aanbevolen in de ECCO-richtlijn; met een interval van 4 maanden (onze studie) versus met een interval van 2-3 maanden (ECCO-richtlijn). Daarom concluderen we dat de monitoring frequentie van laboratoriumparameters verlaagd kan worden naar minder dan elke 4 maanden. Na de introductie van genetische testen voor TMPT, is het voorkomen van thiopurine geïnduceerde beenmergtoxiciteit verminderd.

We speculeren dat een monitoringstrategie met controle van laboratoriumparameters per 6 maanden voldoende is na 1 jaar thiopurine behandeling en dat dit de belasting voor de patiënt en de zorgkosten zal verminderen. Een prospectieve studie in een onpraktisch grote studiepopulatie en meerdere jaren follow-up zou vereist zijn om deze speculaties te bevestigen. Daarnaast is de verwachting dat het vaststellen van laboratoriumtoxiciteit zowel de behandeling als de monitoringfrequentie zal beïnvloeden en dat een prospectief design dit niet zal voorkomen en derhalve niet de vereiste gegevens op zal leveren om deze hypothese te testen. In deze studie zijn patiënten met thiopurine monotherapie bestudeerd, maar het zou ook interessant zijn om de klinische relevantie van laboratoriumtoxiciteit te bestuderen

in patiënten die worden met behandeld met combinatietherapie met een thiopurine en een biological.

Uitdagingen in adalimumab behandeling
Biologicals worden in toenemende mate toegepast in de behandeling van IBD-patiënten, maar de werkzaamheid en tolerantie van deze medicijnen in de dagelijkse klinische praktijk verschillen van de gerapporteerde cijfers uit gerandomiseerde gecontroleerde onderzoeken die vaak onder strikte voorwaarden zijn uitgevoerd in een geselecteerde patiëntenpopulatie. Om gepersonaliseerde behandeling mogelijk te maken is het noodzakelijk om factoren die verband kunnen houden met de effectiviteit, veiligheid en tolerantie van een behandeling te bestuderen in een cohort afkomstig uit de dagelijks klinische praktijk. In **Hoofdstuk 6** beschreven we een prospectieve real-life cohortstudie van 188 CD-patiënten die werden behandeld met de biological adalimumab (ADA) waarbij we de werkzaamheid en tolerantie beoordeelden. In het bijzonder hebben we ons verdiept in mogelijke sekse verschillen in de effectiviteit van de behandeling. Het bleek dat het vrouwelijk geslacht het behandelingssucces van ADA negatief beïnvloedt. Ten eerste hadden vrouwelijke patiënten minder kans op klinische respons of het behouden van respons dan mannelijke patiënten (31% versus 48%). Ten tweede rapporteerden vrouwelijke patiënten meer bijwerkingen zoals huidreacties en infecties en zagen we dat vrouwen vaker de behandeling stopten vanwege bijwerkingen dan mannen. Dit suggereert dat een gepersonaliseerde benadering van vrouwelijke patiënten die starten met ADA een positieve invloed zou kunnen hebben op het behandelsucces. Op andere gebieden binnen de geneeskunde is aangetoond dat het geslacht van de patiënt grote invloed kan hebben op zowel het metabolisme als de werkzaamheid van geneesmiddelen, ook bij behandelingen met biologicals. Hoewel het exacte mechanisme onbekend is, zouden onze resultaten kunnen worden verklaard door geslacht-specifieke fysiologische verschillen in geneesmiddelmetabolisme, lichaamssamenstelling of geslachtshormonen. Behalve fysiologische verklaringen, zijn er ook verschillen tussen mannen en vrouwen in de perceptie van bijwerkingen en de mate waarmee de toegang tot gezondheidszorg wordt gezocht. In een recente systematische review, uitgevoerd door Lie et al, werd geen bewijs gevonden voor sekseverschillen in de werkzaamheid van behandeling met biologicals wanneer werd gekeken naar objectieve metingen zoals uitkomsten van endoscopie. Deze bevinding wekt sterkt de suggestie dat het lagere succespercentage van ADA bij vrouwen grotendeels wordt veroorzaakt door het staken van de therapie als gevolg van bijwerkingen. Het verstrekken van informatie en voorlichting op maat over de mogelijke bijwerkingen zou kunnen inspelen op de verwachtingen van de patiënt over het medicijn en mogelijk het uitvalspercentage door bijwerkingen kunnen verminderen.

Cervicale neoplasie en IBD
Hoewel verschillende onderzoeken suggereren dat IBD-vrouwen een verhoogd risico lopen op cervicale neoplasie, dat wordt veroorzaakt door humaan papillomavirus (HPV), is de rol van IBD ziektekarakteristieken en gebruik van immunosuppressieve therapie in de pathogenese onduidelijk. Vanwege tegenstrijdig bewijs en gebrek aan longitudinale follow-

up in de meeste onderzoeken is het onduidelijk of cervicale screening bij IBD moet worden geïntensiveerd. In **Hoofdstuk 7** hebben we het risico op hooggradige cervicale dysplasie en baarmoederhalskanker (CIN2+) bestudeerd in een groot cohort met longitudinale follow-up waarin we IBD vrouwen hebben vergeleken met een gematcht cohort bestaande uit vrouwen afkomstig uit de algemene Nederlandse populatie. We ontdekten dat de mate waarin CIN2+ werd vastgesteld (detectiepercentage) hoger was in het IBD cohort dan in het gematchte controles, zelfs na correctie voor hun frequentere screeningsgedrag. Dit risico was het hoogst bij vrouwen in de leeftijdsgroep van 35 tot 39 jaar. Roken werd in eerdere studies reeds beschreven als risicofactor, en ook in onze studie was dit een sterke voorspeller voor CIN2+. Echter, het risico van roken op CIN2+ was hoger in IBD vrouwen dan vrouwen van de algemene bevolking wat een gecombineerd effect van IBD en roken zou suggereren op het ontwikkelen van CIN2+. Dit zou patiënten verder moeten aanmoedigen om te stoppen met roken. Blootstelling aan immunomodulatoren en biologicals, geanalyseerd als trichotome variabelen (nooit, <1 jaar blootstelling,> 1 jaar blootstelling), was niet geassocieerd met het ontwikkelen van CIN2+. Belangrijk is dat we aantoonden dat het risico op progressie van een normaal uitstrijkje naar CIN2+ verhoogd was bij IBD vrouwen, en dat IBD vrouwen ook een verhoogd risico liepen op aanhoudende of recidiverende CIN afwijkingen vergelijken met vrouwen uit het gematchte cohort. Deze bevindingen zouden kunnen worden verklaard door een verminderde klaring van hoog-risico HPV infecties, als gevolg van verminderde detectie van oncogene signalen (immunosurveillance) veroorzaakt door chronische systemische inflammatie. Dit wordt ondersteund door het feit dat we geen verschil in voorbijgaande laaggradige CIN1 afwijkingen tussen beide groepen konden vaststellen. Deze bevindingen benadrukken het belang van HPV-vaccinatie en deelname aan het bevolkingsonderzoek baarmoederhalskanker bij alle IBD-vrouwen, ongeacht het gebruik van immunosuppressieve medicatie. Dit wordt ondersteund door het feit dat een HPV-vaccinatie in patienten die immunosuppressieve medicatie gebruiken een normale immuunrespons veroorzaakt**.**

In **Hoofdstuk 8** hebben we het effect van blootstelling aan immunosuppressiva (immunomodulatoren en / of biologische geneesmiddelen) en CIN 2+ ontwikkeling in het IBD-cohort nader onderzocht. Hoewel blootstelling aan immunosuppressiva, uitgedrukt als dichotome variabelen (ooit / nooit blootstelling) geen invloed leek te hebben op de ontwikkeling van CIN2+, toonden we aan dat het risico op CIN2+ wordt verhoogd met elk jaar blootstelling aan immunomodulatoren (HR 1.15 per behandeljaar) of biologicals (HR 1.15 per behandeljaar). Om rekening te houden met het uitgestelde effect van blootstelling aan medicatie en het ontwikkelen van CIN2+ (latentietijd) werden de analyses herhaald met een vertraginsperiode van 6 en 12 maanden, echter dit gaf geen belangrijke verandering in resultaten. De invloed van immunosuppressieve medicatie op de ontwikkeling van CIN2+ zou opnieuw verklaard kunnen worden door een verminderde klaring van hrHPV-infecties veroorzaakt door de (iatrogeen) immuungecompromitteerde status van patiënten. Het is belangrijk dat artsen het belang van HPV-vaccinatie en deelname aan het bevolkingsonderzoek naar baarmoederhalskanker benadrukken in alle IBD-vrouwen. Deze resultaten benadrukken

dat vervolgonderzoek nodig is om te beoordelen of intensievere screening gunstig is voor IBD vrouwen die langdurig worden blootgesteld aan behandeling met immunosuppressiva.

Lokale behandeling van proctitis ulcerosa

Lokale behandeling verdiend de voorkeur in proctitis ulcerosa (UP), een vorm van UC waarbij het laatste gedeelte van de dikke darm ontstoken is, omdat er weinig systemische bijwerkingen zijn. Helaas is het arsenaal aan lokale therapieën beperkt en hebben patiënten met een 5-ASA-refractaire proctitis vaak intensievere behandeling nodig met (lokale) corticosteroïden, immunomodulatoren of biologials die mogelijk gepaard gaan met bijwerkingen en hogere kosten. Vanwege veelbelovende resultaten in eerdere onderzoeken hebben we de effectiviteit van het medicijn tacrolimus onderzocht in zetpilvorm. **Hoofdstuk 9** beschrijft deze multicenter dubbelblinde gerandomiseerde gecontroleerde studie in patiënten met 5-ASA refractaire UP, waarin de helft van de patiënten werd behandeld met tacrolimus zetpillen en de andere helft van de patiënten met beclometason (corticosteroïd) zetpillen, gedurende 4 weken. Uiteindelijk zijn 85 patiënten in de studie geïncludeerd en na 4 weken behandeling toonden tacrolimus- en beclomethason-zetpillen een vergelijkbare klinische respons (60%) en endoscopische respons (40%). Bovendien resulteerden beide behandelingen in histologische genezing van ontsteking en verbeterde kwaliteit van leven. In deze studie werden geen significante verschillen waargenomen in het aantal bijwerkingen tussen tacrolimus en beclometason behandeling. Onze bevindingen impliceren dat zowel tacrolimus- als beclometason zetpillen geschikt zijn als behandeling voor patiënten met 5-ASA refractaire UP. Adequate behandeling van UP is niet alleen belangrijk voor controle van symptomen en behoud van kwaliteit van leven, maar ook om ziekteprogressie te verminderen/voorkomen. Lokale behandeling met tacrolimus of beclometason zou overwogen moeten worden bij 5-ASA-refractaire UP voorafgaand aan step-up naar systemische behandeling met thiopurines of biologials. Voor de toekomst zou het interessant zijn om de effectiviteit en tolerantie van onderhoudsbehandeling met tacrolimus in UP in de praktijk te onderzoeken**.**

Aanbevelingen voor toekomstig onderzoek

Het is een uitdaging om het juiste medicijn voor de juiste patiënt te selecteren in het groeiende doolhof van IBD behandelingen. Adequate behandeling van IBD is essentieel om complicaties van de ziekte op de lange termijn te voorkomen. Ondanks aanzienlijke inspanningen is er nog geen behandeling die tot genezing van IBD leidt. Onderzoek naar nieuwe behandelstrategieën voor IBD is van belang, maar er is zeker nog ruimte voor verbetering met de huidige beschikbare behandelingen.

Naast het effectief behandelen van de ontstekingsreactie, dienen ook inspanningen geleverd te worden om de monitoring van respons en bijwerkingen te verbeteren en adequate optimalisatiestrategieën toe te passen om therapie falen te voorkomen en zo de klinische uitkomsten te verbeteren.

Vroege (preventieve) TDM-geleide thiopurinetherapie lijkt veelbelovend om falen van thiopurine therapie te voorkomen en toekomstige prospectieve studies zouden zich moeten richten op de (lange termijn) effectiviteit, toxiciteit en kosteneffectiviteit van deze strategie, al dan niet gecombineerd met genetische testen voorafgaand aan de behandeling. Idealiter wordt volledige genotypering van alle patiënten verricht voorafgaand aan behandeling met thiopurines om zo genetische variaties aan het licht te brengen die het metabolisme van thiopurine beïnvloeden. Op deze manier zou uiteindelijk voorkomen kunnen worden dat patiënten worden blootgesteld aan geneesmiddelen die niet effectief of schadelijk voor hen zijn vanwege ernstige toxiciteit en daarom baat zouden hebben bij alternatieve therapieën. Aangezien TDM in wezen het effect van alle genetische variaties en inadequate dosering omvat, is het de vraag of uitgebreid farmacogenetische testen voorafgaand aan behandeling uiteindelijk zal voorkomen dat patiënten nog routinematige monitoring voor toxiciteit moeten ondergaan.

Er wordt een vergelijkbaar voordeel gezien van optimalisatiestrategieën met LDTA en TG bij patiënten bij wie conventionele thiopurines falen. Vanwege hun gunstige eigenschappen op het thiopurine metabolisme zou de werkzaamheid van eerstelijnsbehandeling met LDTA of TG verder moeten worden bestudeerd, bij voorkeur in een head-to-head trial.

Een ander aspect om te onderzoeken zijn thiopurine-analogen die zich specifiek richten op de Rac1-signaleringsroute om T-cel apopotose te stimuleren waarbij de enzymatische routes geassocieerd met toxiciteit worden omzeilt. Geoptimaliseerde immunosuppressieve medicijnen kunnen relevante therapeutische implicaties bieden indien dit wordt bevestigd in klinische onderzoeken. Hoewel de overgrote meerderheid van nieuwe immunosuppressieve medicijnen systemische therapieën zijn, blijven lokale therapieën onderontwikkeld. Om gepersonaliseerde behandeling mogelijk te maken voor patiënten met distale vormen van IBD, is het aanbevolen om ook (nieuwe) lokale therapieën te onderzoeken.

Bij (ernstig) actieve IBD wegen de risico's van medische behandeling over het algemeen op tegen mogelijke ziekte-gerelateerde complicaties, maar de risico's van langdurige immunosuppressieve behandeling mogen niet naar de achtergrond verdwijnen. Aangezien cervicale neoplasie bij IBD-vrouwen toeneemt, moeten toekomstige studies gericht zijn op het onderzoeken van de persistentie van hrHPV infecties en de rol bij cervicale carcinogenese. Dit zou kunnen leiden tot strategieën waarbij het gebruik van immunosuppressieve medicatie (tijdelijke) wordt verminderd om zodat het virus geklaard kan worden en de cervicale carcinogenese wellicht te onderbreken of zelfs om te keren, voordat de laesies niet meer reversibel zijn. Naast baarmoederhalskanker zijn oncogene HPV-infecties ook geassocieerd met hoofd-, nek- en anogenitale kankers. De huidige schattingen geven aan dat 5,2% van alle kankers HPV-geassocieerd zijn, en het risico op HPV-infectie gelijk is voor mannen en vrouwen. Derhalve zouden toekomstige studies ook de impact van immunosuppressieve blootstelling bij andere HPV-gerelateerde kankers en de werkzaamheid van HPV-vaccinatie bij mannelijke IBD patiënten moeten onderzoeken.

Een belangrijke stap in de vertaling van onze resultaten naar de klinische praktijk is de ontwikkeling van een 'voorspellingstool' die gepersonaliseerde behandeling mogelijk maakt. Vanwege de complexiteit van IBD zijn er verschillende factoren die de ontwikkeling van een gepersonaliseerde behandelstrategie belemmeren. Het is onwaarschijnlijk dat gerandomiseerde studies het bewijs zullen leveren voor een dergelijke strategie, aangezien deze onderzoeken strikte in- en exclusiecriteria voor patiënten hanteren en daarmee niet de klinische praktijk vertegenwoordigen. Het consistent verzamelen van longitudinale klinische gegevens en biomaterialen in landelijke databases, waaronder de Nederlandse IBD biobank (PSI) zoals beschreven in dit proefschrift, zou de ontwikkeling van een therapeutische strategie voor de individuele IBD-patiënt mogelijk kunnen maken. Toekomstig onderzoek zou de verdere ontwikkeling van gepersonaliseerde behandelstrategieën moeten onderzoeken, bij voorkeur in het kader van internationale samenwerking. Een veelbelovend nieuw onderzoeksgebied om complexe klinische gegevens te analyseren in combinatie met gegevens van biomaterialen is dynamische systeemanalyse, waarmee 'realtime' geïndividualiseerde voorspelling van ziekte en behandelingsresultaten mogelijk is.

Conclusies

IBD blijft een complexe ziekte met een grote interindividuele variantie in ziektegedrag en behandelingsrespons. Dit proefschrift draagt bij aan de huidige inzichten op het gebied van immunosuppressieve behandeling en biedt het verschillende mogelijkheden om IBD zorg te verbeteren. Het combineren van deze bevindingen met klinische gegevens en biomaterialen zou kunnen leiden tot de ontwikkeling van meer op maat gemaakte behandelstrategieën en zal hopelijk in de nabije toekomst ook realiteit worden.

APPENDIX

LIST OF ABBREVIATIONS

ABBREVIATIONS

5-ASA	5-aminosalicylic acid
6-MP	6-mercaptopurine
6-MMPR	6-methylmercaptopurine ribonucleotides
6-TG	6-thioguanine
6-TGN	6-thioguanine nucleotides
6-TGTP	6-thioguanosine 5' triphosphate
ADA	adalimumab
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AP	alkaline phosphatase
AZA	azathioprine
BIO	biologics
BMI	body mass index
CD	Crohn's disease
CDAI	Crohn's disease activity index
CI	confidence interval
CIN	cervical intraepithelial neoplasia
CRP	C-reactive protein
CRC	colorectal cancer
CTCAE	Common Terminology Criteria for Adverse Events
CYP	cytochrome P450
FBC	full blood count
γ-GT	gamma-glutamyltransferase
Hb	haemoglobin
HR	hazard ratio
HGPRT	hypoxantine-guanine phosphoribosyl transferase
HPV	human papillomavirus
hrHPV	high-risk types of human papillomavirus
IBD	inflammatory bowel disease
IBDI	inflammatory bowel disease indeterminate
IBDQ	inflammatory bowel disease questionnaire
IBDU	inflammatory bowel disease unclassified
IRR	incidence rate ratios
IQR	interquartile range
LDTA	low dose thiopurine allopurinol combination therapy
LTs	liver tests
MCV	mean corpuscular volume
IM	immunomodulators
IS	immunosuppressive drugs

MP	mercaptopurine
MRCP	magnetic resonance cholangio- pancreatography
MRI	magnetic resonance imaging
N	number
OR	odds ratios
PC	platelet count
PSI	Parelsnoer Insitute, Dutch IBD biobank
RBC	red blood cells
RCT	randomized controlled trial
SC	subcutaneously
SD	standard deviation
SDR	standardized detection rates
TDM	therapeutic drug monitoring
TNF-a	tumor necrosis factor alpha
TPMT	thiopurine S-methyltransferase
TG	tioguanine
UC	ulcerative colitis
ULN	upper limit of normal
UP	ulcerative proctitis

APPENDIX

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APPENDIX

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   No Superiority of Tacrolimus Suppositories vs Beclomethasone Suppositories in a Randomized Trial of Patients With Refractory Ulcerative Proctitis.
   Clinical Gastroenterology and Hepatology. 2020 Jul;18(8):1777-1784.

2. **Kreijne JE**, de Vries AC, de Veer RC, Bouma G, Dijkstra G, Voskuil MD, West R, van Moorsel SAW, de Jong DJ, de Boer NK, van der Woude CJ. On behalf of the initiative on Crohn and Colitis (ICC).
   Limited added value of laboratory monitoring in thiopurine maintenance monotherapy in inflammatory bowel disease patients.
   Alimentary Pharmacolology & Therapeutics. 2020 Jun;51(12):1353-1364.

3. **Kreijne JE**, de Veer RC, de Boer NK, Dijkstra G, West R, Moorsel SAW, de Jong DJ, van der Woude CJ, de Vries AC; of the Dutch Initiative on Crohn, Colitis (ICC).
   Real-life study of safety of thiopurine-allopurinol combination therapy in inflammatory bowel disease: myelotoxicity and hepatotoxicity rarely affect maintenance treatment.
   Alimentary Pharmacolology & Therapeutics. 2019 Aug;50(4):407-415.

4. Meijer B, **Kreijne JE**, van Moorsel SAW, Derijks LJJ, Bouma G, Mulder CJJ, Wong DR, van der Woude CJ, van Bodegraven AA, de Boer NKH.
   6-methylmercaptopurine induced leukocytopenia during thiopurine therapy in IBD patients.
   Journal of Gastroenterology and Hepatology. 2017 Jun;32(6):1183-1190.

5. Lie MRLK, **Kreijne JE**, van der Woude CJ.
   Sex is associated with adalimumab side effects and drug survival in patients with Crohn's disease.
   Inflammatory Bowel Disease. 2017 Jan;23(1):75-81.

6. **Kreijne JE**, Seinen ML, Wilhelm AJ, Bouma G, Mulder CJ, van Bodegraven AA, de Boer NK.
   Routinely established skewed thiopurine metabolism leads to a striking high rate of early therapeutic failure in patients with inflammatory bowel disease.
   Therapeutic drug monitoring 2015 Dec; 37(6):797-804.

7. **Kreijne JE***, Goetgebuer RL*, Aitken CA, Dijkstra G, Hoentjen F, de Boer NK, Oldenburg B, van der Meulen AE, Ponsioen CJJ, Pierik MJ, van Kemenade FJ, de Kok IMCM, Siebers AG,

Manniën J, van der Woude CJ, de Vries AC. *Increased risk of high-grade cervical neoplasia in women with inflammatory bowel disease: a case-controlled cohort study.*
Submitted

8. **Kreijne JE***, Goetgebuer RL*, Erler, N, de Boer NK, Aitken CA, Dijkstra G, Hoentjen F, Oldenburg B, van der Meulen AE, Ponsioen CJJ, Pierik MJ, van Kemenade FJ, Siebers AG, van der Woude CJ, de Vries AC.
Drug exposure and cervical neoplasia in women with inflammatory bowel disease.
Manuscript in preparation

Other publications

9. **Kreijne JE**, Lie MR, Vogelaar L, van der Woude CJ.
Practical Guideline for Fatigue Management in Inflammatory Bowel Disease.
Journal of Crohns and Colitis. 2016 Jan;10(1):105-11.

10. **Kreijne JE***, van der Giessen J*, Verhaar AP, Peppelenbosch MM, de Vries AC, van der Woude CJ, Fuhler GM.
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11. Harinck F, van Putten PG, **Kreijne JE**, Bruno MJ, van der Woude CJ, de Vries AC.
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12. Kanis SL, **Kreijne JE,** van der Woude CJ.
Commentary on " Pregnancy outcomes in women with inflammatory bowel disease following exposure to thiopurines and antitumor necrosis factor drugs: A systematic review with meta-analysis".
Human & Experimental Toxicology. 2017 Sep;36(9):993-994.
** Shared first authorship*

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PHD PORTFOLIO

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Name PhD student: Joany E. Kreijne
PhD period: December 2014 – Augustus 2019
Erasmus MC department: Gastroenterology and Hepatology
Promotor: Prof. dr. C.J. van der Woude
Co-promotores: Dr. A.C. de Vries and dr. K.H.N. de Boer

Courses and workshops

	Year	Workload
Journal clubs, department of Gastroenterology and Hepatology, Erasmus MC, Rotterdam	2014-2017	60 hours
BROK course, Consultatiecentrum Patiëntgebonden onderzoek (CPO), Erasmus MC, Rotterdam	2015	24 hours
EndNote workshop, Erasmus MC, Rotterdam	2015	6 hours
Systematic Literature Retrieval in PubMed workshop, Erasmus MC, Rotterdam	2015	6 hours
Systematic Literature Retrieval in other databases workshop, Erasmus MC, Rotterdam	2015	6 hours
Basic Introduction on SPSS, Molecular medicine postgraduate school, Rotterdam	2015	24 hours
Biomedical English Writing Course, Molecular medicine postgraduate school, Rotterdam	2016	15 hours
Introduction in GraphPad Prism, Molecular medicine postgraduate school, Rotterdam	2016	10 hours
Indesign workshop, Molecular medicine postgraduate school, Rotterdam	2017	4 hours
Photoshop & Illustrator workshop, Molecular medicine postgraduate school, Rotterdam	2017	8 hours
Survival analysis course, Molecular medicine postgraduate school, Rotterdam	2017	12 hours
English Biomedical Writing and Communication, Erasmus MC, Rotterdam	2017	84 hours
Integrity in scientific research, Dept. of Medical ethics and Philosophy, Erasmus MC, Rotterdam	2016	16 hours

Oral presentations

	Year	Workload
Routinely established skewed thiopurine metabolism leads to a strikingly high rate of early therapeutic failure in patients with inflammatory bowel disease. Nederlandse Vereniging voor Gastro-enterologie voorjaarscongres, Veldhoven, The Netherlands	2014	12 hours
Common genetic predisposition between fatigue-related syndromes and Inflammatory Bowel Disease. Nederlandse Vereniging voor Gastro-enterologie voorjaarscongres, Amsterdam, The Netherlands	2016	12 hours
Monitoring of laboratory parameters during thiopurine maintenance therapy in patients with inflammatory bowel disease: an unnecessary burden? Nederlandse Vereniging voor Gastro-enterologie najaarscongres, Veldhoven, The Netherlands	2017	12 hours

Tacrolimus suppositories as induction therapy for refractory ulcerative proctitis: a randomised controlled trial. 13th congress of European Crohn's and Colitis Organisation, Vienna, Austria. **Awarded top 10 best abstract presentation**	2018	24 hours
Tacrolimus suppositories as induction therapy for refractory ulcerative proctitis: a randomised controlled trial. Digestive Disease Week, Washington D.C., United States of America	2018	24 hours
Hoe vaak op controle met thiopurine? ICC dag, Initiative on Crohn and Colitis, Amsterdam, the Netherlands	2019	12 hours

Poster presentations

	Year	Workload
Routinely established skewed thiopurine metabolism leads to a strikingly high rate of early therapeutic failure in patients with inflammatory bowel disease. 9th congress of European Crohn's and Colitis Organisation, Barcelona, Spain	2014	12 hours
Common genetic predisposition between fatigue-related syndromes and Inflammatory Bowel Disease. 11th congress of European Crohn's and Colitis Organisation, 2016 Amsterdam, The Netherlands	2016	12 hours
Monitoring of laboratory parameters during thiopurine maintenance therapy in patients with inflammatory bowel disease: an unnecessary burden? United European Gastroenterology Week, 2017, Barcelona, Spain.	2017	12 hours
LDTA Drug survival of thiopurine-allopurinol combination therapy in a real-life population based cohort. 13th congress of European Crohn's and Colitis Organisation, 2018, Vienna, Austria	2018	12 hours
Monitoring of laboratory parameters during thiopurine maintenance therapy in patients with inflammatory bowel disease: an unnecessary burden? 13th congress of European Crohn's and Colitis Organisation, 2018, Vienna, Austria	2018	12 hours

Attended (inter)national conferences

	Year	Workload
9th congress of European Crohn's and Colitis Organisation, Copenhagen, Denmark	2014	28 hours
Half-yearly Meeting of the Dutch Association of Gastroenterology, Veldhoven, the Netherlands	2015	12 hours
10th congress of European Crohn's and Colitis Organisation, Barcelona, Spain	2015	28 hours
11th congress of European Crohn's and Colitis Organisation, Amsterdam, the Netherlands	2016	28 hours
Half-yearly Meeting of the Dutch Association of Gastroenterology, Veldhoven, the Netherlands	2017	12 hours
12th congress of European Crohn's and Colitis Organisation, Barcelona, Spain	2017	28 hours
United European Gastroenterology Week, 2017, Barcelona, Spain	2017	28 hours
12th congress of European Crohn's and Colitis Organisation, Barcelona, Spain	2018	28 hours
Half-yearly Meeting of the Dutch Association of Gastroenterology, Veldhoven, the Netherlands	2018	12 hours
Digestive Disease Week, Washington D.C., United States of America	2018	28 hours

Grants

Mrace pilot project 2016, Doelmatigheidsonderzoek Erasmus MC Project: Predict the unpredictable": 2 yearly or 4 yearly monitoring of laboratory parameters in thiopurine treated patients with inflammatory bowel disease?

Attended seminars

	Year	Workload
9th Y-ECCO Career Workshop, Barcelona, Spain	2015	6 hours
2nd Post-ECCO meeting, Utrecht, the Netherlands	2015	4 hours
Science for science conference, National scientific agenda, Den Haag, the Netherlands	2015	6 hours
10th Y-ECCO Career Workshop, Amsterdam, the Netherlands	2016	6 hours
10th Young-ICC symposium, Amsterdam, the Netherlands	2016	4 hours
3rd Post-ECCO meeting, Amsterdam, the Netherlands	2016	2 hours
HPV-research day, RIVM, Zeist, the Netherlands	2016	8 hours
ICC dag, Amsterdam, the Netherlands	2017	6 hours
11th Y-ECCO Career Workshop, Barcelona, Spain	2017	4 hours
11th Young-ICC symposium, Amsterdam, the Netherlands	2017	4 hours
4th Post-ECCO meeting, Amsterdam, the Netherlands	2017	2 hours
8ste Lagerhuisdebat Leverziekten, Utrecht, the Netherlands	2017	2 hours
ICC dag, Amsterdam, the Netherlands	2019	6 hours

Memberships

Dutch Society of Gastroenterology (NVGE)

European Crohn's and Colitis Organisation (ECCO)

Peer review activities

American Journal of Gastroenterology, Cochrane systematic review, Journal of Crohn's and Colitis

Educational activities and lecturing

	Year	Workload
Lecture 'IBD en vermoeidheid', landelijke bijeenkomst voor patienten met IBD, CCUVN, Rotterdam, the Netherlands	2015	12 hours
Lecture 'abdominal pain, inflammatory bowel disease', first year students curriculum Medicine, Erasmus University Rotterdam, Rotterdam	2014-2018	10 hours
Supervising systematic review project, minor students Medicine, Erasmus University Rotterdam, Rotterdam	2016	8 hours
ECCO teaching program, Basic principles of management of IBD, Nicosia, Cyprus	2016	20 hours
ECCO teaching program, Surgical management of IBD, Nicosia, Cyprus	2016	12 hours
Supervising scientific research project Rozanne de Veer, masterstudent Medicine, Erasmus University Rotterdam, Rotterdam	2016	40 hours

APPENDIX

DANKWOORD

DANKWOORD

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APPENDIX

ABOUT THE AUTHOR

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Joany Ellis Kreijne was born on June 27th 1987 in Amersfoort, The Netherlands. She attended secondary school at the Nieuwe Eemland College in Amersfoort and graduated in 2005. She then studied Health Sciences at the Vrije Universiteit and completed the first year. In 2006 she started medical school at the VU University medical Center. During her clinical rotations she did an elective in Hepatology at the University Hospital Birmingham, United Kingdom. The groundwork for the thesis was laid in 2013 during her research rotation at the department of Gastroenterology and Hepatology of the VU University Medical Center, when she researched implications of thiopurine metabolism variations for patients with inflammatory bowel disease under the supervision of dr. K.H.N. de Boer. After obtaining her medical degree in 2013, she first worked as a medical doctor at the deparment of Pulmonary Medicine and the department of Internal Medicine, Gastroenterology and Hepatology at the Alkmaar Medical Center. In December 2014 she started her PhD at the department of gastroenterology and hepatology of the Erasmus MC Medical Center Rotterdam, under the supervision of prof. dr. C.J. van der Woude, dr. K.H.N de Boer and dr. A.C. de Vries, ultimately resulting in this dissertation. From September 2018 onwards she started her training in gastroenterology and hepatology in the Spaarne Gasthuis in Haarlem and Hoofddorp. She will continue her residency at the Amsterdam University Medical Centers.