ASSESSMENT OF TNFα –BLOCKING THERAPY IN INFLAMMATORY BOWEL DISEASE PATIENTS IN DEEP REMISSION

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ACADEMIC DISSERTATION

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To my family
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications:


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*I: John Wiley and Sons; II, IV Elsevier; III Walter Kluwer Health
ABBREVIATIONS

5-ASA 5-aminosalicylate
15D 15-dimensional
ACCENT I A Crohn’s Disease Clinical Trial Evaluating Infliximab in a New Long Term Treatment Regimen
ACCENT II A Crohn’s Disease Clinical Trial Evaluating Infliximab in a New Long Term Treatment Regimen in Patients With Fistulizing Crohn’s Disease
ACT Active Ulcerative Colitis Trial
ADA adalimumab
ANCA anti-neutrophil cytoplasmic antibodies
ASCA anti-Saccharomyces cerevisiae antibodies
ATA anti-adalimumab antibodies
ATG16L1 autophagy-related protein 16-1 gene
ATI anti-infliximab antibodies
AUC area under the curve
CARD caspase-activating recruitment domain
CBir1 anti-flagellin antibody
CD Crohn’s disease
CDAI Crohn’s disease activity index
CDEAS Crohn’s disease endomicroscopic activity score
CDEIS Crohn’s disease endoscopic index of severity
CHARM Crohn’s disease trial of the fully Human antibody Adalimumab for Remission Maintenance
CI confidence interval
CRP C-reactive protein
CT computed tomography
CZP certolizumab
DBE double-balloon enteroscopy
DR deep remission
EGD esophagogastroduodenoscopy
ELISA enzyme-linked immunosorbent assay
EQ-5D European quality of life – five dimensions
ESR erythrocyte sedimentation rate
EXTEND extend the safety and efficacy of adalimumab through endoscopic healing
F fecal
FC fecal calprotectin
FL lactoferrin
GAB anti-goblet cell antibodies
GI gastrointestinal
GLM golimumab
HBI Harvey-Bradshaw index
HRQoL health-related quality of life
IBD inflammatory bowel disease
IBDQ inflammatory bowel disease questionnaire
IBDU inflammatory bowel disease unclassified
IBS irritable bowel syndrome
IFN interferon
ABSTRACT

Background

The inflammatory bowel diseases (IBDs), Crohn’s disease (CD), ulcerative colitis (UC), and inflammatory bowel disease unclassified (IBDU) are chronic inflammatory conditions of the gastrointestinal tract characterized by remissions and exacerbations. Recently, the therapeutic targets have aimed towards mucosal healing (MH), which is associated with less hospitalization and surgery and also with better quality of life. Moreover, during the era of TNFα-blocking therapy, deep remission (DR), meaning clinical remission with MH, has been the most desirable target for therapy in IBD. We constructed a study to evaluate how often patients on TNFα-blocking maintenance therapy actually achieve DR and also to evaluate the disease course, predictive factors, impact of histological remission on relapse risk, and response to retreatment in patients with IBD in DR after cessation of TNFα-blocking therapy. Fecal calprotectin (FC) concentration has been shown to be a useful surrogate marker for MH during TNFα-blocking therapy in IBD, and high FC levels seem to indicate a risk of IBD relapse during clinical remission. Our aim was to evaluate whether a normal FC after induction therapy with TNFα-blocking agents predicts the outcome of IBD patients during maintenance therapy and whether elevated FC concentration after cessation of TNFα-blocking therapy can predict clinical or endoscopic relapse in asymptomatic patients.

Patients and methods

To evaluate the achievement of DR in Study II, data were collected retrospectively at eight gastroenterological units in various parts of Finland for all UC, CD, and IBDU patients treated with scheduled TNFα-blocking therapy for at least 11 months, after which they underwent an ileocolonoscopy during 2010 and 2011. A total of 252 patients with IBD (183 patients with CD, 62 with UC, and 7 with IBDU) were recruited. At the time of ileocolonoscopies, clinical disease activity was assessed by physicians’ global assessment (PGA) scores and endoscopic activity in CD by the simple endoscopic score for Crohn’s disease (SES-CD), and in UC and IBDU by the Mayo endoscopic score. To evaluate histological activity, biopsies were taken from the most severely affected areas during ileocolonoscopies, and findings were graded as normal or active inflammation.

Prospective, multicenter Studies III and IV explored the relapse rate, predictive factors, impact of histological remission on relapse risk, response to retreatment, and capacity of FC to predict relapse in IBD patients in DR after discontinuing TNFα-blocking therapy. These studies included 52 patients (17 CD, 30 UC, 5 IBDU) in clinical, endoscopic, and FC-based remission after at least one year of TNFα-blocking therapy. Patients were recruited between February 2010 and June 2012 at nine gastroenterological
units. Clinical and endoscopic remission and relapse were defined according to the Harvey-Bradshaw index (HBI) and SES-CD in CD and Mayo score in UC and IBDU. After discontinuing TNFα-blocking therapy, all patients were followed up with clinical assessment and blood tests every four weeks for six months and thereafter every second month up to 12 months or until the relapse. Endoscopic and histological assessment of disease activity was performed at 4 and 12 months and at relapse. In the event of a clinical relapse with endoscopically active disease or minor clinical symptoms, but severe endoscopic relapse, TNFα-blocking therapy was reinitiated. Patients were asked to provide a stool sample for FC measurement prior to every visit and to fill out questionnaires on quality of life at baseline, at time of ileocolonoscopies, and at relapse.

Study I was constructed to evaluate the predictive value of FC measurement after induction of TNFα-blocking therapy. This study comprised 60 IBD patients (34 CD and 26 UC), who were treated for active luminal disease and had an elevated FC level at baseline and a documented FC concentration after induction with TNFα-blocking agonists. After the induction therapy, patients were divided into two groups according to the postinduction FC level. Clinical disease activity was assessed with HBI in CD and with partial Mayo score in UC at baseline, after induction with TNFα-blocking agonists, and at one year.

Results

Among 252 patients receiving TNFα-blocking maintenance therapy, 67% were in clinical remission and 48% in DR at the time of ileocolonoscopy (Study II). Clinical remission was achieved in 63% of CD patients and 75% of UC patients, whereas DR was achieved in 43% of CD patients and 62% of UC patients. No significant difference in achieving clinical remission emerged between CD and UC patients (p = 0.072), but DR was significantly more common in UC patients than in CD patients (p = 0.007). In this study, FC measurements were available for 163 patients at the time of ileocolonoscopy. Median FC level was significantly lower in patients in DR than in the others (50 μg/g, range 1–722 vs. 288 μg/g, range 6–4190, p < 0.0001).

When exploring the outcome after cessation of TNFα-blocking therapy (Study III), 33% patients relapsed after a median follow-up time of 13 (range 12-15) months. Ten patients experienced clinical and endoscopic relapse, five patients clinical relapse with mild endoscopic activity, and two CD patients severe endoscopic relapse. The relapse rate was equal in both CD and UC (p = 0.896). Based on univariate analysis, no specific predictive factors were associated with the relapse. Reassuringly, the retreatment with TNFα-blocking agents was effective in 94% of patients.

In Study I, the FC cut-off value of 139 μg/g was optimized as the best cut-off for predicting one-year clinical relapse, with a sensitivity of 72% and a specificity of 80% (AUC 0.838 (0.724–0.952)). Smoking (OR 1.19, 95% CI 4.32–0.33, p = 1), gender (OR 0.33; 95% CI 0.11–1.00, p = 0.064), or chosen...
TNFα-blocking agents (OR 0.35; 95% CI 0.10–1.23, p = 0.147) did not significantly affect the outcome. An FC decline of more than 88% during TNFα-blocking induction therapy predicted clinical remission with a sensitivity of 87% and, a specificity of 65% (AUC 0.771 (0.652–0.890), p <0.001). After discontinuing TNFα-blocking therapy, patients relapsing showed constantly elevated FC levels for a median of 94 (13–317) days before the relapse (Study IV). FC levels were significantly higher than levels at baseline two (p = 0.0014), four (p = 0.0056), and six (p = 0.0029) months before endoscopic relapse. More importantly, once FC was found to be elevated, it remained elevated until the relapse. Constantly normal FC concentrations or only transiently elevated FC concentrations during the follow-up were highly predictive for clinical and endoscopic remission. Normal FC concentrations in patients with remission were associated with histological remission. Mild or moderate histological inflammation at the time of cessation of TNFα-blocking therapy did not have an impact on relapse risk.

**Conclusions**

DR is achievable in up to half of IBD patients on TNFα-blocking maintenance therapy in everyday clinical practice. A concomitant histological remission is achievable relatively often. Despite achieving DR, discontinuing TNFα-blocking therapy was considered reasonable for only about half of our patients. When TNFα-blocking therapy was ceased in IBD patients in DR, up to 67% remained in clinical remission during the 12-month follow-up. Moreover, the majority of these patients also remained in endoscopic remission. In relapers, the response to restart of TNFα-blocking agonist appeared effective and well tolerated. A normal FC after TNFα-blocking induction therapy predicts sustained clinical remission in the majority of patients with active luminal disease with scheduled treatment, and it may also predict endoscopic remission. In addition, FC seems to be a useful surrogate marker for predicting relapse in patients with IBD and identifying patients requiring a close follow-up in clinical practice.
INTRODUCTION

Inflammatory bowel diseases (IBDs), including Crohn’s disease (CD) ulcerative colitis (UC), and inflammatory bowel disease unclassified (IBDU), are chronic idiopathic inflammatory disorders affecting the gastrointestinal tract (Abraham et al. 2009). While UC and IBDU affect only the colon, CD may affect the whole gastrointestinal tract. The etiology and pathogenesis of these conditions remain somewhat obscure. The most widely accepted hypothesis is that these conditions arise from interactions between immunoregulatory, genetic, and environmental factors. In IBD, bowel damage is induced by an uncontrolled activation of both innate and adaptive immunity due to an imbalance between pro-inflammatory cytokines. The natural history of IBD is characterized by repeated episodes of inflammation and ulceration of the bowel, which may lead to complications that lower the quality of life and increase the need for hospitalization, surgery and escalation of therapy (Langholz et al. 1999, Peyrin-Birule et al. 2010).

Treatment decisions for IBD have usually been based on disease severity, localization, and extent. Recent years, management of IBD has changed markedly, as the tumor necrosis factor alpha (TNFα) antagonists infliximab (IFX), adalimumab (ADA), and most recently golimumab (GLM) have become available. Earlier, the treatment goal for IBD has been to achieve clinical response or remission and normalization of laboratory parameters. Recently, mucosal healing (MH) and prevention of extraintestinal complications have been regarded as important treatment goals, in managing IBD. MH is assessed by endoscopy and is thought to be an important prognostic factor for the efficacy of treatment in IBD associated with fewer hospitalizations and less surgeries (Lichtenstein et al. 2002, 2004, Schnitzler et al. 2009). As endoscopies are time-consuming and unpleasant for patients, alternative methods to assess MH have emerged. In IBD, the levels of fecal calprotectin (FC), an inflammatory product of the intestinal mucosa, correlates closely with endoscopic and histological grading of disease activity (Roseth et al. 1999, Sipponen et al. 2008a). A normalized FC level has been shown to be a useful predictor of MH in IBD patients, and high FC levels may indicate a risk of relapse during clinical remission (Tibble et al. 2000b, Costa et al. 2005, D’Inca et al. 2008, Garcia-Sanchez et al. 2010).

TNFα antagonists induce and maintain remission in patients with moderate to severe CD (Rutgeerts et al. 2006, Colombel et al. 2007, Rutgeerts et al. 2012) and UC (Rutgeerts et al. 2005, Sandborn et al. 2012a). Although TNFα-blocking therapy has been used in clinical practice for over a decade, scant data have appeared on the prevalence of concomitant clinical remission and MH during maintenance therapy with anti-TNFα agents. This information is essential when considering the possibility of discontinuation of TNFα-blocking therapy in IBD patients. In clinical practice, the decision on whether to continue or discontinue TNFα-blocking therapy in IBD patients in DR is still based on assessment of the patient’s individual risks and benefits.
Existing guidelines have concluded that due to limited evidence no recommendations can be made on when and in whom to discontinue TNFα-blocking therapy after having achieved clinical remission (Dignass et al. 2010, D’Haens et al. 2011, Dignass et al. 2012a). This and the chronic nature of IBD may lead to long-term maintenance therapy with TNFα antagonists, raising questions about safety and economic issues. A few studies, mainly on CD, have been published on the duration of remission after discontinuation of TNFα-blocking therapy, and potential risk factors for relapse (Waugh et al. 2010, Louis et al. 2012, Steenholdt et al. 2012, Molnar et al. 2013, Rismo et al. 2013, Farkas et al. 2013). None of these studies have assessed endoscopic activity during the follow-up, nor have they determined the optimal monitoring strategy after discontinuation of TNFα-blocking therapy.

The objective of this thesis was to establish how often DR can be achieved in IBD patients on TNFα-blocking therapy in everyday clinical practice and to assess the relapse rate and predictive factors of relapse after cessation of maintenance therapy with TNFα-blocking agents in IBD patients in DR. Additionally, this thesis aimed to evaluate the role of FC in predicting long-term response to TNFα-blocking therapy and its capacity to predict a clinical or endoscopic relapse after discontinuation of TNFα-blocking therapy.
REVIEW OF THE LITERATURE

The first description of IBD was provided by British physician Samuel Wilks in 1895, who recognized UC from bacterial dysentery, and also described a case report of a woman with a transmural inflammation of the colon and terminal ileum, resembling ileoceecal CD (Kirsner 1988). CD, also known as terminal ileitis, regional enteritis, granulomatous ileitis, hyperplastic ileitis, chronic interstitial enteritis, and chronic ulcerative ileitis, was first described by Polish surgeon Antoni Leśniowski in 1904. Nonetheless, CD is named after an American gastroenterologist Burrill Crohn, who described fourteen patient cases with regional enteritis in 1932, and later that year published the case series as "Regional ileitis: a pathologic and clinical entity" with his colleagues Leon Ginzburg and Gordon Oppenheimer (Crohn et al. 1932).

CD is a chronic transmural inflammatory disease of the gastrointestinal (GI) tract that can affect any part of the GI tract. CD is commonly associated such complications as abscesses, fistulas, and strictures. Unlike CD, UC is a nontransmural inflammatory disease that is restricted to the colon. Both conditions are characterized by phases of remission and episodes of relapse. In some patients the disease may be chronically active, meaning a continuously active inflammation of the gut (Baumgart and Sandborn 2007).

1 Etiology and pathogenesis of inflammatory bowel disease

Although knowledge of immunological mechanisms has improved greatly in recent years, the etiology of IBD remains largely unknown. Research indicates that an individual’s genetic susceptibility, external environment, intestinal microbial flora, and immune responses are all involved and functionally integrated in the pathogenesis of IBD (Danese et al. 2006).

1.1 Genetics

A positive family history is the single greatest risk factor for IBD. In population-based studies, the proportion of IBD patients having a positive family history varies from 2% to 20% (Gaya et al. 2006). The pooled concordance in monozygotic twins has also been demonstrated to be 36% (Rosenstiel et al. 2009). Evidence also suggests ethnic aggregation of IBD, with higher rates of IBD among Jewish people than in any other ethnic group. The modern era of IBD genetic research began in 2001 with the discovery of encoding caspase-activating recruitment domain 15 (CARD15), also known as nucleotide-binding oligomerization domain 2 (NOD2), the first susceptibility gene for CD (Ogura et al. 2001). The NOD2 gene codes for a protein that was originally described as an intracellular receptor recognizing the muramyl dipeptide (MDP), a conserved motif present in peptidoglycan from both Gram-positive and -negative bacteria (Inohara et al. 2003). MDP stimulation induces autophagy, which controls bacterial
replication and antigen presentation and modulates both innate and adaptive immune responses (Cooney et al. 2010, Travassos et al. 2010). Mutations of this gene are strongly associated with CD affecting ileum and with stricturing CD (Gaya et al. 2006). CARD15/NOD2 seems to also be a disease-modifier gene for CD. Furthermore, the Th17 and IL-23 pathway is well established in the pathogenesis of IBD, with susceptibility gene loci IL23R, IL12B, JAK2, and STAT3 having been identified in both UC and CD (Anderson et al 2011, Brand et al 2009). Defects in the function of IL-10 have also been associated with CD and UC (Tremelling et al. 2007). Moreover, genetic analyses have shown an indispensable role for autophagy in immune responses in IBD and have reported two autophagy-related genes, ATG16L1 and IRGM (Rioux et al. 2007, Hampe et al. 2007, McCarroll et al. 2008). Recent studies have brought the number of IBD-associated gene loci to 163, of which 110 are associated with both diseases, 30 are CD-specific and 23 are UC-specific (Jostins et al. 2012).

1.2 Environmental factors

There is no doubt that several environmental factors, such as smoking, diet, viruses, bacteria, drugs, especially nonsteroidal anti-inflammatory drugs (NSAIDs), geography, social stress, and a psychological component, play important roles in the pathogenesis of IBD. Among these risk factors, smoking remains the most widely studied and documented (Cosnes 2004, Mahid et al. 2006, Lakatos et al. 2007, Higuchi et al. 2012, Lakatos et al. 2013). Smoking increases the risk of developing CD and worsens its course, raising exacerbation rates and the need for steroids and immunosuppressants and promoting complications and reoperations (Cosnes et al. 2002, Johnson et al. 2005). Contrary to its effect on CD, subsequent studies have confirmed the protective effect of heavy smoking on the development of UC-related relapses (Cosnes 2004). Diet has been hypothesized to play an important role in the pathogenesis of IBD. The dietary factors that most likely affect IBD development are fat, carbohydrates, macronutrients, and protein, in particular saturated fatty acids, fiber, omega-3 and omega-6 fatty acids and refined sugar (Cruber et al. 2012). High intakes of fat, polyunsaturated fatty acids, omega-6 fatty acids, and meat have been shown to increase the risk of CD and UC, while high intakes of fruits and fiber decrease CD risk, and a high intake of vegetables decreases the risk of UC (Hou et al. 2011). Vitamin deficiencies in general and vitamin D deficiency in particular often occur in patients with IBD (Andreassen et al. 1997). Bendix-Struve and co-workers (2010) demonstrated that vitamin D3 modifies T-cell proliferation and increases IL-6 levels in CD patients, hence having an impact on the pathogenesis of CD.

1.3 Immune response

Available evidence suggests that dysfunctions of the innate and adaptive immune pathways contribute to the aberrant intestinal inflammatory response in patients with IBD. The innate immune system is responsible for the early immune response, providing a nonspecific, rapid defence against pathogens with monocytes, macrophages, neutrophils, dendritic cells,
natural killer (NK) cells, and the complement system (Medzhitov and Janeway 2000). This innate immune system is inborn and not tailored to any particular immunological challenge. This form of immunity is initiated by the recognition of microbial antigens, which is provided by pattern-recognition receptors (PRRs), including membrane-associated toll-like receptors (TLRs) on the cell surface and NOD-like receptors in the cytoplasm (Abreu et al. 2005). Studies have demonstrated that the behavior of the cells mediating innate immunity and the expression and function of both TLRs and NOD proteins are altered significantly in individuals with IBD (Bonen et al. 2003, Abraham and Cho 2006).

The adaptive – recognized also as the specific – immune system is slower and tailored by T- and B-lymphocytes. Over the past years, most studies have focused on the role of abnormal adaptive immune responses in the pathogenesis of IBD. CD and UC represent clearly distinct forms of gut inflammation; CD has long been considered to be driven by a Th1 response, and UC has been associated with a nonconventional Th2 response (Cobrin et al. 2005, Targan et al. 2005). Most recently, also Th17 cells, regulated by IL-23, have been shown to be involved in the gut inflammatory response in IBD (Geremia et al. 2012). The activation of Th1 and Th17 cells in CD provides abundant interleukins (ILs), and transforming growth factor β by antigen presenting cells and macrophages. These cells increase the secretion of the pro-inflammatory cytokines IL-2, IL-17, interferon (IFN) -γ, and TNFα, leading to intestinal inflammation. The cytokines, in turn, feed into a self-sufficient cycle and stimulate antigen-presenting cells, macrophages, fibroblasts, and endothelial cells to produce TNFα, IL-1, IL-6, IL-8, IL-12, and IL-18 (Collison et al. 2010, Engel and Neurath 2010, Franke et al. 2010). On contrast, in UC, atypical NK T-cells release higher amounts of the Th2 cytokine IL-13 than T-cells from controls or CD patients (Heller et al. 2005). In addition, defects in regulatory T-cell function and in T-cell apoptosis may occur (Brown and Mayer 2007).

1.4 Microbiota

The whole human gut microbiome consists of approximately 1150 bacterial species, with each individual host having approximately 160 species (Qin et al. 2010). The gut microbiome is established within the first two weeks of life and thereafter usually remains remarkably stable. The GI microbiome of healthy humans is dominated by four major bacterial phyla: Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria (Morgan et al. 2012). Some studies examining the gut flora in CD and UC in both inflamed and noninflamed segments have found a significantly reduced biodiversity in the fecal microbiome of IBD patients relative to healthy controls (Joossens et al. 2011). Other studies have described the microbiota as more unstable in IBD patients than in healthy individuals (Andoh et al. 2011). In IBD patients the microbiota is characterized by a relative lack of Firmicutes and Bacteroidetes, and an overrepresentation of enterobacteria (Sartor 2008). Also a reduction in Clostridium spp. and an increase in Escherichia coli, especially an
adherent and invasive E. coli (AIEC), invasive Fusobacterium nucleatum, and mucolytic bacteria such as Ruminococcus gnavus and Ruminococcus torques and Mycobacterium avium paratuberculosis (MAP) have been reported in IBD (Martinez et al. 2008, Feller et al. 2007, Khor et al. 2011). However, the role of MAP in IBD pathogenesis remains obscure (Bull et al. 2003). Patients with IBD have a compromised mucus layer and an epithelial surface that is densely coated with bacteria; the abundant presence of Ruminococcus strains in IBD mucosa raises the possibility that such microbes may contribute to the barrier defect observed in IBD, although whether their presence is causal or correlative is unclear (Khor et al. 2011).

2 Epidemiology

A north–south gradient in Europe exists, with higher incidence rates of IBD in northern countries, but the incidence in southern and eastern Europe is increasing (Burisch et al. 2013). Recently, the prevalence of UC seems to be decreasing, whereas the prevalence of CD is increasing due to earlier onset of the disease and a low mortality rate (Loftus et al. 2007). The highest incidence and prevalence rates can be found in developed and modernized countries, with annual incidence of up to 16.3/100,000 and prevalence of 213/100,000 (Loftus 2004, Baumgart and Carding 2007, Bernstein and Shanahan 2008). In a recent European cohort study (EccoEpiCoM), a very high incidence of UC was found on the Faroe Islands, being 31.8/100,000 in UC and 83.1/100,000 in IBD (Burisch et al. 2014). In Finland, the prevalence of IBD has increased nearly threefold during the past 15 years, being 291/100,000 for UC and 124/100,000 for CD (Manninen et al. 2010). Distinct north-south and west-east gradients exist within Europe, with the highest incidence rates in northern and western countries (Burisch et al. 2014). An increase during the last decade in the incidence of both UC and CD has been demonstrated in a Finnish study locally (Manninen et al. 2010), but the incidence seems to be more stable nationwide (Jussila et al. 2012). A clear north-south gradient has also been observed (Jussila et al. 2013).

3 Disease localization and disease behavior

Typical presentation of CD includes discontinuous involvements of various portions of the GI tract from the mouth to the anus and potential development of disease complications such as abscesses, fistulae, or strictures. Typically, at diagnosis, about one-quarter of patients have both ileal and colonic disease, another quarter have only colonic disease, and about half have only terminal ileitis. Less than 10% of CD patients have ileal involvements out of reach of ileocolonoscopy or involvements in the proximal small bowel or upper GI tract (Baumgart and Sandborn 2007). Localization of the disease remains exceedingly stable over time, but the disease behavior may change, typically from inflammatory to stricturing or penetrating (Louis et al. 2001, Cosnes et al. 2002). Patients with ileal CD are

UC tends to begin in the rectum and extend proximally, affecting the bowel in a continuous fashion. A classification by the Montreal Working Group divides UC into proctitis (E1), left-sided colitis, also known as distal colitis (E2), and extensive colitis (E3) and also takes into consideration disease behavior and age at onset (Silverberg et al. 2005). Typically, at diagnosis, 40–50% of patients have proctitis, 30–40% left-sided colitis and up to 25–30% pancolitis (Conrad et al. 2014). Disease can progress and spread over time. Left-sided colitis or proctitis may extend to pancolitis in up to 53% of patients (Moum et al. 1999). Although UC is restricted to the colon by definition, nonspecific mucosal inflammation in the terminal ileum (“backwash ileitis”) is found in 10–20% of UC patients (Conrad et al. 2014).

4 Diagnosis

No single test for diagnosis of IBD exists. In 1997, Lennard-Jones and Shivananda defined widely accepted macroscopic and microscopic criteria for diagnosing IBD. Macroscopic tools include physical, endoscopic, and radiological examination, and less frequently, examination of surgical specimens. In CD, the diagnosis is based on noncontinuous and often granulomatous inflammation of the gut, while in UC the inflammation is typically continuous with a decreasing gradient of inflammation from the distal to the proximal colon. Diagnosis is currently based on a combination of clinical presentation, endoscopic features, histological findings, radiological manifestations, surgical findings, and serological abnormalities (Van Assche et al. 2010a, Dignass et al. 2012b). In 5–10% of IBD patients, no definite diagnosis of CD or UC can be made when only the colon is affected. For these particular cases the term “inflammatory bowel disease unclassified” (IBDU) is used (Satsangi et al. 2006). The term “indeterminate colitis” refers to a pathological-anatomical diagnosis describing a colectomy specimen with overlapping features of CD and UC (Price 1978, Satsangi et al. 2006).

4.1 Clinical presentation

Clinical presentation of CD. A heterogeneity of manifestations, a potentially insidious onset, the presence of overlapping features with other IBD, and a presentation without GI symptoms, can make diagnosis of CD extremely difficult. Chronic or nocturnal diarrhea is the most common symptom of CD, affecting up to 85% of patients (Sands 2004). Abdominal pain occurs in approximately 70% and weight loss in 60% of patients. In colonic CD, bloody and/or mucous stools occur in about half of patients (Lennard-Jones and
Shivananda 1997). Fever, rectal bleeding and fatigue may also present. Approximately 75% of patients with large bowel CD develop perianal pathology, including skin tags, deep ulcers, fissures, fistulae, abscesses, blind sinus tracts, and strictures at some point during the disease course (Warren 2004). Clinical signs also include pallor, cachexia, an abdominal mass or tenderness, and aphthous ulcers in the oral cavity. Associated extraintestinal features may include peripheral arthropathy, axial arthritis, uveitis, episcleritis, erythema nodosum, pyoderma gangrenosum, or hepatobiliary disease such as primary sclerosing cholangitis. Extraintestinal manifestations are most common in colonic CD (Van Assche et al. 2010a). In children, anemia, fever, failure of growth, or delayed development of secondary sex characteristics may be observed (Langholz et al 1997). Although the onset is typically insidious, occasionally CD presents in a fulminant manner at its onset or with the presence of toxic megacolon (Swan et al. 1998).

Clinical presentation of UC. Clinically, UC is characterized by loose stool or diarrhea and chronic abdominal pain. Patients with active disease also present with rectal urgency, tenesmi, mucus or blood in stool, nocturnal and postprandial defecation, or even constipation (Lennard-Jones and Shivananda 1997, Dignass et al. 2012b). The clinical picture mainly depends on the extent of bowel involvement, disease activity, and extraintestinal manifestations and complications. Inflammatory arthropathies and primary sclerosing cholangitis (PSC) are the most common and important extraintestinal manifestations in UC. PSC is diagnosed in about 2–10% of UC patients and occurs occasionally with autoimmune hepatitis (overlap syndrome). As in CD, other extraintestinal manifestations involve skin (erythema nodosum, pyoderma gangrenosum), eyes (episcleritis, uveitis), and bones (osteoporosis) (Van Assche et al. 2013).

4.2. Laboratory findings

Laboratory features are not specific markers for IBD. They detect inflammatory processes (elevated erythrocyte sedimentation rate [ESR], C-reactive protein [CRP], FC) or deficiencies due to malnutrition (iron deficiency, anemia) and may help to assess disease activity as well as complications. Anemia and thrombocytosis are the most common changes seen in IBD patients, especially in CD. In addition, ESR and CRP may be elevated and albumin reduced. Stool testing for pathogenic bacteria, particularly Clostridium difficile, and other parasites is necessary to exclude infectious colitis. The most frequently studied serological markers in IBD are antineutrophil-cytoplasmic antibodies (ANCA), directed against Candida albicans, and antibodies against mannann of Saccharomyces cerevisiae (ASCA). Perinuclear (pANCA) or atypical ANCA (xANCA) can be found in 50–70% of UC patients and in less than 10% of CD patients. ANCA positivity and a negative test for ASCA are more likely to indicate UC than CD (Conrad et al. 2002). In patients with IBDU, combined determination of ANCA and ASCA may provide a definitive diagnosis. Another serological marker, specific for UC, is anti-goblet cell antibodies (GAB), occurring in 15–28% of UC patients. In an ideal setting, GAB is highly specific for UC (Conrad et al. 2006). Furthermore, antibody responses towards E. coli outer membrane
porin C (anti-ompC), a CD-related bacterial sequence from *Pseudomonas fluorescens* (anti-12), and towards a flagellin CBir1 (anti-CBir1) are seen in approximately half of CD patients and only about 10% of UC patients (Mow et al. 2004). Anti-CBir1 expression has been shown to be associated with small bowel disease as well as with penetrating and stricturing disease (Vernier et al. 2004). These antibody responses may play a role in subtyping IBD patients or in predicting disease course, but because of their inaccuracy, these tests are of little use in clinical practice and have limited value in monitoring disease activity.

### 4.3 Endoscopy

Endoscopy plays an essential role in the diagnosis, management, prognosis, and surveillance of IBD. Initial endoscopy should intubate the terminal ileum, and in clinical practice this is achieved in approximately 85% of ileocolonoscopies. During the endoscopy multiple biopsy specimens should be taken from all segments of the bowel. CD is characterized by a discontinuous and ulcerous transmural inflammation, often involving the ileocaecal region, but can be detected in the whole digestive tract, typically with the involvement of the terminal ileum and cecum. Small, deep aphthous ulcers or longitudinal ulcers, anal lesions, and a cobblestone appearance of the ileum are the most common features of CD (Nikolaus and Schreiber 2007). By contrast, UC typically presents with continuous, uniform inflammation that extends proximally from the rectum. The line between inflamed and normal areas is usually clear and may occur abruptly, especially in distal disease. Occasionally, in total colitis a “backwash” ileitis occurs. In children, rectal sparing has been described prior to treatment, whereas in adults this is more likely due to a topical treatment (Rajwal et al. 2004, Odze et al. 1993). In severe, active colitis ileocolonoscopy may lead to an increased risk of bowel perforation, and therefore, flexible sigmoideoscopy may be preferred, with ileocolonoscopy reserved for later use (Van Assche et al. 2010a). Surveillance colonoscopies can be improved by spraying dyes that highlight subtle changes in the architecture of the colonic mucosa. Chromoendoscopy enhances mucosal detail and submucosal vascular pattern and aids in discriminating between neoplastic and non-neoplastic changes, based on surface crypt architecture (pit pattern). Chromoendoscopy has a sensitivity of 83.3% and a specificity of 91.3% in detecting intraepithelial neoplasia (Wu et al. 2012). However, endoscopies are time-consuming, expensive, require bowel preparation, and are unpleasant for patients.

In adult IBD, no specific recommendations for esophagagastroduodenoscopy (EGD) exist, but it is often performed only on patients with upper GI symptoms, like dyspepsia or abdominal pain. However, some experts suggest that it should be performed at least once on all newly diagnosed CD patients (Hommes and van Deventer 2004). By contrast, EGD is mandatory in pediatric IBD patients with growth failure problems, to differentiate between UC and CD and to confirm a diagnosis of CD (Castellaneta et al. 2004, Crocco et al. 2012).
Earlier, only radiographical techniques were available for examinations of the entire small bowel. Recently, small bowel wireless capsule endoscopy (WCE), magnetic resonance enterography (MRE) and double-balloon enteroscopy (DBE) have made it possible to examine the entire small bowel. WCE is a device-assisted enteroscopy, which has been designed to examine the entire small bowel and to visualize mucosal inflammation. This novel technique is useful in assessment of the extent and severity of small bowel CD as well as distinguishing between patients with CD and UC. In clinical practice WCE has replaced conventional radiology (small bowel follow-through; SBFT), barium enteroclysis (SBE), computed tomography (CT), and push enteroscopy in estimating disease extent, but it is still limited by its cost and inability to provide tissue samples or therapy. In patients with established CD, the risk of small bowel capsule retention is increased, particularly in those with a history of obstructive symptoms or known intestinal stenosis (Hoog et al. 2012). Therefore, contraindications for WCE are suspected or diagnosed intestinal stenosis or obstruction. DBE is a device-assisted enteroscopy technique for reaching lesions throughout the entire small bowel (Yamamoto et al. 2001). The scope may be inserted orally or anally depending on which segment of small bowel needs to be examined. The advantages of DBE compared with SBE include the evaluation of atypical lesions, the ability to obtain biopsies for histopathology, and the potential for therapeutic intervention. To date, however, the availability of DBE has been limited, and it should be reserved for situations in which tissue samples are mandatory or dilatations of strictures are required.

4.4 Radiological techniques

Current imaging standards to examine the small bowel comprise computed tomography (CT) and magnetic resonance enterography (MRE). Both techniques can determine disease activity and extension based on wall thickness and increased intravenous contrast enhancement. Because of the increased cancer risk by ionizing radiation later in life, CT is not suitable for repeated use, and conventional radiology or CT should be replaced by alternative methods particularly in children. CT and MRE are imaging techniques with the highest accuracy for diagnosis of ileal and penetrating CD, but for diagnosis of terminal ileitis in CD patients, these techniques are inferior to ileocolonoscopy (Horsthuys et al. 2008, Van Assche et al. 2010a). In many centers, either magnetic resonance enterography or enterolysis has replaced radiation techniques in assessment of CD lesions in the small bowel. These procedures provide information on disease activity, localization, and extension and detect extramural complications such as abscesses, fistulas, and sacroilitis. Importantly, because of an absence of ionizing radiation, MRE can be performed repeatedly and is thus suitable for follow-up of CD patients. Abdominal ultrasound (US) by an experienced operator may provide information on the extent of small bowel or colonic inflammation or possible complications. However, high interobserver variability and difficulty in visualization of deep bowel segments are significant drawbacks (Van Assche et al. 2010a). The use of US in clinical practice in most countries is still restricted.
4.5 Histology

The histological examination of endoscopic biopsies or surgical specimens remains a key step in IBD diagnosis and differential diagnosis, particularly in the differentiation of UC from CD and other non-IBD conditions (Van Assche et al. 2010a). In the initial ileocolonoscopy, multiple biopsies should be taken from each segment of the colon and also from the terminal ileum. In approximately 10–20% of patients with UC, the inflammation may extend to the terminal ileum (backwash ileitis). The diagnostic value of terminal ileum biopsies is highest in patients with known or suspected CD (McHugh et al. 2007).

In CD, the typical histological findings in mucosal biopsy specimens comprise focal crypt architectural abnormalities with the presence of lymphocytes or plasma cells, granulomas, and mucin preservation at active sites (Jerkins et al. 1997, Van Assche et al. 2010a). The granuloma in CD is defined as a collection of epithelioid histiocytes (monocyte/macrophage cells). The presence of a granuloma is not a requirement for the diagnosis of CD. Additional features frequently present are focal chronic inflammation without crypt atrophy, focal cryptitis, aphthoid ulcers, disproportionate submucosal inflammation, neural hypertrophy with increased intraepithelial lymphocytes, and proximal location of ulceration and architectural distortion (Magro et al. 2013). The transmural character of CD inflammation can only be visible in surgical specimens. In UC distorted crypt architecture with crypt branching and atrophy and an irregular villous architecture are more common than in CD (Seldenrijk et al. 1991, Surawicz et al. 1994, Jenkins et al. 1997). Typically, the mucosal inflammation is proportionate, but may occasionally spread into the superficial part of the submucosa. The inflammatory infiltrate is composed of lymphocytes, plasma cells, and neutrophils, causing cryptitis, defined as the presence of neutrophils within crypt epithelium, and crypt abscesses, defined as the presence of neutrophils within crypt lumina. Crypt abscesses are more common in UC (41%) than in CD (19%). Plasma cells are typically observed between the base of the crypts and the muscularis mucosae (basal plasmacytosis). This feature is helpful in differentiation between a first attack of UC (63%) and infectious colitis (6%), but not CD (62%) (Seldenrijk et al. 1991, Schumacher et al. 1994, Surawicz et al. 1994). The inflammation may cause mucin depletion of the epithelium, a less important diagnostic feature as it can also be found in infectious colitis and CD (McCormick et al. 1990, Surawicz et al. 1994). Depending on the degree of inflammatory activity the surface may become eroded. Chronic features of IBD include Paneth cell metaplasia (especially in left-sided colitis), presence of inflammatory pseudopolyps, hypertrophy of the muscularis mucosae, and rarely identified submucosal fibrosis (Gramlich et al. 2007). Granulomas are not found in biopsies of patients with UC.

5 Health-related quality of life (HRQoL)

The concept of “quality of life” describes the general well-being of an individual or a society. Assessment of health-related quality of life (HRQoL)
may shed light on the chronic illness experience, its effects on health outcomes, and the overall effect of a disease on a person’s ability to enjoy life. HRQoL measurement helps to identify the most appropriate therapy for individuals (Sajid et al. 2008). On the population level HRQoL measurement can be used for monitoring overall health and any changes in this, as well as the effects of social and health policies. In recent years, HRQoL measurement has become an important tool in medical science and is also an essential outcome in cost-effectiveness and cost-utility analyses.

There are two main types of HRQoL instruments, generic and disease-specific. Generic instruments are typically used in comparing health status among patients with different health states, conditions, and diseases. However, the most important function of these instruments is to enable comparisons between populations with different diseases (Cramer et al. 2002). The most commonly used generic HRQoL instruments are the Medical Outcomes Study Short-Form (SF-36), EuroQoL (EQ-5D), Sickness Impact Profile (SIP), Nottingham Health Profile (NHP), Psychological General Well-Being (PGWB), and 15D. The latter is a standardized and self-administered measure of HRQoL among adults. The 15D comprises of 15 dimensions: breathing, mental function, speech (communication), vision, mobility, usual activities, vitality, hearing, eating, elimination, sleeping, distress, discomfort and symptoms, sexual activity, and depression, each rated on a 5-point scale. The 15D score varies from 0 (decreased) to 1 (no problems in any dimension) (Sintonen 1994). The most commonly used HRQoL instruments in IBD is the Inflammatory Bowel Disease Questionnaire (IBDQ), a validated, standardized 32-item questionnaire, which has been translated into over 40 languages (ip.mcmaster.ca/questionnaires). The questionnaire focuses on four different aspects of life: digestive symptoms, social function, emotional status, and systemic symptoms, each including 5–12 questions. Responses range from 1 to 7. A total IBDQ score ranges from 32 to 224, with a higher score indicating a better quality of life (Guyatt et al. 1989, Pallis et al. 2004). Other commonly used HRQoL instruments in IBD are the Rating Form of IBD Patient Concerns (RFIPC) and the Cleveland Clinic IBD Scale.

6 Assessment of disease activity

Global disease activity assessment in clinical practice relies on clinical history and a combination of clinical, serological, endoscopic, and radiological findings. Clinical trials focusing on treatment response and long-term outcome have revealed the need for standardized and qualitative disease activity indices based on clinical symptoms or findings or their combinations.

6.1 Clinical activity

Various activity indices exist. The score most commonly used in clinical trials for CD is the Crohn’s disease activity index (CDAI), which comprises one serological (hematocrit) and seven clinical variables (number of liquid stools, abdominal pain, general well-being, extraintestinal features, antidiarrheal
medication, abdominal mass, and body weight) (Best et al. 1976). CDAI score ranges from 0 to approximately 650, with CDAI score < 150 indicating inactive disease and CDAI score > 450 severe disease (Sostegni et al. 2003). Clinical response, a common endpoint in many clinical trials, is suggested to be defined as a reduction of ≥100 CDAI points or in some studies ≥ 70 CDAI points (Van Assche et al. 2010a). The CDAI score is not commonly used in everyday clinical practice because it is rather complex and time-consuming to calculate and necessitates keeping a 7-day diary of symptoms. In addition, it is not suitable for patients with extensive ileocolonic resection, stoma, or symptoms caused by strictures or fistula (Sostegni et al. 2003). Further, endoscopic findings and CDAI score correlate poorly, with CDAI underestimating the inflammatory activity detected by endoscopy (Sipponen et al. 2008a). For paediatric CD patients, a pediatric Crohn’s disease activity index (PCDAI) has been developed (Hyams et al. 1991).

In clinical trials, a simple index of CD activity, better known as the Harvey-Bradshaw index (HBI), is often used to determine disease activity. It includes only symptoms and findings from the previous 24 hours (five variables; Table 1) (Harvey and Bradshaw 1980). Clinical remission is often defined as HBI ≤4 or <4 and clinical relapse HBI ≥8 (Best 2006, Sandborn et al 2002). Other clinical activity indices used in clinical trials are summarized in Table 2.

The most commonly used activity indices for UC are modifications of Truelove and Witt’s criteria known as the Mayo score and the Lichtiger score (Truelove and Witts 1955, Lichtiger et al. 1994). The Mayo score is most commonly used, and it combines both clinical and endoscopic findings (Table 3). Scores range from 0 to 12 points, with a higher score indicating more severe disease. Clinical remission is defined as a Mayo score of 0 and clinical response as a decrease of ≥ 3 points from baseline (Schroeder et al. 1987, D’Haens et al. 2007). The noninvasive nine-point Mayo score, known as the partial Mayo score, has been found to indicate clinical activity as well as the full Mayo scores (Lewis et al. 2008).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. General well-being</td>
<td>very well=0, slightly below par=1, poor=2, very poor=3, terrible=4</td>
</tr>
<tr>
<td>B. Abdominal pain</td>
<td>none=0, mild=1, moderate=2, severe=3</td>
</tr>
<tr>
<td>C. Number of liquid stools per day</td>
<td>number of stools</td>
</tr>
<tr>
<td>D. Palpable abdominal mass</td>
<td>no=0, dubious=1, definite=2, definite and tender=3</td>
</tr>
<tr>
<td>E. Complications</td>
<td>arthralgia, uveitis, erythema nodosum, pyoderma gangraenous, aphthous ulcers, anal fissure, new fistula, abscess (score 0 = no, 1 = yes)</td>
</tr>
</tbody>
</table>

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**Table 2.** Clinical activity indices used for Crohn’s disease.

<table>
<thead>
<tr>
<th>Clinical index</th>
<th>Reference</th>
<th>Variables rated</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDAI, Crohn’s Disease Activity Index</td>
<td>Best et al. 1976</td>
<td>diarrhea frequency, abdominal pain, general well-being, use of anti-diarrheal medications, abdominal mass, extraintestinal features, hematocrit, weight</td>
</tr>
<tr>
<td>PCDAI, Pediatric Crohn’s Disease Activity Index</td>
<td>Hyams et al. 1991</td>
<td>abdominal pain, diarrhea, general well-being, weight, height, abdominal findings mass/tenderness, perirectal disease, extraintestinal manifestations, hematocrit, erythrocyte sedimentation rate, albumin</td>
</tr>
<tr>
<td>HBI, Harvey-Bradshaw Index</td>
<td>Harvey and Bradshaw 1980</td>
<td>diarrhea frequency, abdominal pain, general well-being, abdominal mass, extraintestinal features</td>
</tr>
<tr>
<td>van Hees Index, (Dutch Index)</td>
<td>van Hees et al. 1980</td>
<td>body mass index, abdominal mass, sex, temperature, stool consistency, previous resection, extraintestinal manifestations, s-albumin, erythrocyte sedimentation rate</td>
</tr>
<tr>
<td>Oxford Index</td>
<td>Myren et al. 1984</td>
<td>abdominal pain, diarrhea/blood and mucus in stool, perianal involvement, fistulae, other complications, abdominal mass, tenderness, wasting, temperature, hemoglobin</td>
</tr>
<tr>
<td>Cape Town Index</td>
<td>Wright et al. 1985</td>
<td>abdominal pain, stool consistency, well-being, complications (perianal or systemic), fever, abdominal mass, weight, temperature, hemoglobin</td>
</tr>
<tr>
<td>PDAI, Perianal Disease Activity Index</td>
<td>Irvine 1995</td>
<td>discharge of fistulae, pain/restriction of activities, restriction of sexual activity, type of perianal disease, degree of induration</td>
</tr>
<tr>
<td>Short CDAI</td>
<td>Thia et al. 2011</td>
<td>abdominal pain, diarrhea frequency, general well-being</td>
</tr>
</tbody>
</table>
Table 3. Mayo Score (Truelove and Witts 1955).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Stool frequency (patient diary)</td>
<td>0: normal number of stools for the patient</td>
</tr>
<tr>
<td></td>
<td>1: 1–2 stools more than normal</td>
</tr>
<tr>
<td></td>
<td>2: 3–4 stools more than normal</td>
</tr>
<tr>
<td></td>
<td>3: ≥5 stools more than normal</td>
</tr>
<tr>
<td>B. Rectal bleeding (patient diary)</td>
<td>0: no blood seen</td>
</tr>
<tr>
<td></td>
<td>1: streaks of blood with stool less than half the time</td>
</tr>
<tr>
<td></td>
<td>2: obvious blood with stool most of the time</td>
</tr>
<tr>
<td></td>
<td>3: blood alone passed</td>
</tr>
<tr>
<td>C. Findings of endoscopy</td>
<td>0: normal or inactive disease</td>
</tr>
<tr>
<td></td>
<td>1: mild disease</td>
</tr>
<tr>
<td></td>
<td>2: moderate disease</td>
</tr>
<tr>
<td></td>
<td>3: severe disease</td>
</tr>
<tr>
<td>D. Physician’s Global Assessment</td>
<td>0: normal</td>
</tr>
<tr>
<td></td>
<td>1: mild disease</td>
</tr>
<tr>
<td></td>
<td>2: moderate disease</td>
</tr>
<tr>
<td></td>
<td>3: severe disease</td>
</tr>
</tbody>
</table>

Partial (clinical) Mayo Score = A + B + D; Endoscopic Mayo Score = C; Total Mayo Score = A + B + C + D.

6.2 Endoscopic activity

6.2.1 Endoscopic scoring in Crohn’s disease

In CD, the most frequently used scores are the Crohn’s disease endoscopic index of severity (CDEIS), the simplified index SES-CD (simple endoscopic score for Crohn’s disease), and the Rutgeerts score (to evaluate anastomosis after ileocolic resection) (Table 4). The CDEIS was developed at the end of the 1980’s by the French group d’Etude des Affections Inflammatoires digestivesis (Mary and Modigliani 1989). The CDEIS is validated and has become the gold standard for the assessment of endoscopic activity of CD. However, it is complex and time-consuming, subject to interobserver variation, and concentrates on the presence of ulcers, with a range from 0 to 44 points (Sostegni et al. 2003). The threshold for endoscopic remission has been set at CDEIS <6, with other criteria for response (a decrease in CDEIS >5), complete endoscopic remission (CDEIS <3), and mucosal healing (absence of ulcers). The complexity of CDEIS makes it unsuitable for clinical practice. It also correlates poorly with clinical activity (Cellier et al. 1994).

To simplify endoscopic assessment, a simple endoscopic score for Crohn’s disease (SES-CD) has been developed and validated more than 10 years ago (Dapero et al 2002). It is easier and faster to calculate and is therefore more suitable for clinical practice. It is known to correlate well with CDEIS. SES-CD is based on four variables scored in the five ileocolonic segments (Table 5). The ileum is scored for the full segment that it is visualized, excluding the ileocecal valve and the ileocecal anastomosis. The right colon includes the ileocecal valve, the cecum, and the ascending colon up to the hepatic flexure. The transverse colon includes the bowel segment from the hepatic flexure to
the splenic flexure, and the left colon the descending and sigmoid colon. The rectum is defined as the bowel distal to the rectosigmoid junction. The score ranges from 0 to 60, with a higher score indicating more severe inflammation. The most often used cut-off for remission is 0-2, mild inflammation 3-6, moderate inflammation 7-15, and severe inflammation ≥ 16 (Moskovitz et al. 2007, Sipponen et al. 2008a). Thus, remission as also been defined as SES-CD 0-3 (Schoepfer et al. 2010). Both CDEIS and SES-CD seem to overestimate colonic disease and underestimate ileal disease as well as severe but short segmental colonic disease (Sipponen et al. 2008a).

For postsurgical assessment of disease activity, the Rutgeerts score is considered the gold standard (Sostegni et al. 2003). The Lemann score, also known as the Crohn’s disease digestive damage score (Pariente et al. 2012) is a newly developed score that aims to identify CD patients at risk for rapid damage progression who would most likely benefit from early induction of immunosuppressive or anti-TNF therapy. This score is time-consuming and is therefore suitable mainly for clinical studies. New endoscopic techniques, such as high-definition endoscopy, magnification endoscopy, filter endoscopy, and chromoendoscopy, enable the endoscopist to obtain real-time in vivo histology views during endoscopy for the generation of optical biopsies. Recent studies have used endomicroscopy for in vivo assessment of endoscopic activity in IBD. Neumann and coworkers (2012) developed the first endomicroscopic activity index to determine the severity of inflammation in CD, the Crohn’s Disease Endomicroscopic Activity Score (CDEAS). Furthermore, the Watson score, based on cell shedding seen in endomicroscopy, has been developed to assess local barrier dysfunction in vivo for predicting clinical relapses in CD (Kiesslich et al. 2012). However, validation of these new scores is lacking.
<table>
<thead>
<tr>
<th>Endoscopic index</th>
<th>Reference</th>
<th>Variables or remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDEIS</td>
<td>Mary et al. 1989</td>
<td>deep ulcers, superficial ulcerations, surface affected by disease (cm), surface affected by ulcerations (cm)</td>
</tr>
<tr>
<td>SES-CD</td>
<td>Dapero et al. 2004</td>
<td>size of ulcers, ulcerated surface, affected surface, presence of narrowing</td>
</tr>
<tr>
<td>Rutgeerts Score</td>
<td>Rutgeerts et al. 1990</td>
<td>i0: no lesions in the distal ileum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i1: ≤5 aphthous lesions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i2: ≥5 aphthous lesions with normal mucosa between the lesions, or skip areas of larger lesions or lesions restricted to ileocolonic anastomosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i3: aphthous ileitis with diffusely inflamed mucosa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i4: diffuse inflammation with large ulcers, nodules, or narrowing</td>
</tr>
<tr>
<td>CDEAS</td>
<td>Neumann et al. 2012</td>
<td>Analysis of mucosa by using fluorescein-aided confocal laser endomicroscopy in IBD. Not validated.</td>
</tr>
<tr>
<td>Watson Score</td>
<td>Kiesslich et al. 2012</td>
<td>Assess local barrier dysfunction in CD in vivo. May be used to predict clinical relapse. Not validated.</td>
</tr>
</tbody>
</table>
### Table 5. Simple endoscopic score for Crohn’s disease (SES-CD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>Size of ulcers</strong></td>
<td>none</td>
</tr>
<tr>
<td><strong>Ulcerated surface</strong></td>
<td>none</td>
</tr>
<tr>
<td><strong>Affected surface</strong></td>
<td>unaffected</td>
</tr>
<tr>
<td><strong>Presence of narrowing</strong></td>
<td>none</td>
</tr>
</tbody>
</table>


#### 6.2.2 Endoscopic scoring in Ulcerative Colitis

There are many scoring systems developed for assessment the endoscopic activity in UC (Table 6). The Mayo endoscopy subscore (Schroeder et al. 1987) has been most commonly used, providing endoscopic subscore from 0 to 3 and defining mucosal healing as a score of ≤1 (normal mucosa or loss of vascular pattern, but no mucosal friability). Recently, the Ulcerative Colitis Endoscopic Index of Severity (UCEIS) was developed as the first validated index of endoscopic severity. The final UCEIS score includes three components (vascular pattern, bleeding, erosions and ulcers), each with precise definitions and three or four levels of severity. The UCEIS score seem to give precise overall assessment of endoscopic severity of UC, although the threshold for MH has not yet been determined.
## Table 6. Endoscopic disease activity indices used for UC.

<table>
<thead>
<tr>
<th>Endoscopic index</th>
<th>Reference</th>
<th>Variables or remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayo Endoscopy</td>
<td>Schroeder et al. 1987</td>
<td>findings of flexible sigmoidoscopy: erythema, vascular pattern, friability, erosions, ulcers, spontaneous bleeding 4-point scale</td>
</tr>
<tr>
<td>Subscore</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baron Score</td>
<td>Baron et al. 1964</td>
<td>vascular pattern, bleeding 4-point scale</td>
</tr>
<tr>
<td>Modified Baron Score</td>
<td>Feagan et al. 2005</td>
<td>vascular pattern, friability, bleeding, ulcers 5-point scale</td>
</tr>
<tr>
<td>Sutherland Index</td>
<td>Sutherland et al. 1987</td>
<td>friability, bleeding 4-point scale</td>
</tr>
<tr>
<td>UCIDS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sutherland Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcerative colitis disease activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Powell-Tuck Index (St Mark’s index)</td>
<td>Powell-Tuck et al. 1987</td>
<td>20-point scale with 2 additional points for endoscopic findings (bleeding) Mainly based on clinical parameters</td>
</tr>
<tr>
<td>Rachmilewitz Index</td>
<td>Rachmilewitz et al. 1989</td>
<td>granulation, vulnerability, vascular pattern, mucosal damage 12-point scale</td>
</tr>
<tr>
<td>UCEIS</td>
<td>Travis et al. 2012</td>
<td>vascular pattern, bleeding, erosions and ulcers 3- to 4-point scale</td>
</tr>
<tr>
<td>Ulcerative Colitis Endoscopic Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>of Severity</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 6.2.3 Endoscopic activity and mucosal healing

Endoscopy with biopsies is the golden standard for assessment of intestinal inflammation and its extension. Also follow-up endoscopies may be needed when speculating on the uncertainty of disease localization, activity, or even diagnosis. With the advent of WCE and especially DBE, more complete assessment of the small bowel in CD is possible.

The assessment of MH is relevant in both clinical practice and clinical trials. Since the 1960’s, clinical studies on UC have suggested a more favorable outcome after corticosteroid therapy in UC patients achieving a clinical and endoscopic remission than in those only achieving a clinical remission (Wright et al. 1966). Until the late 1990’s, studies on CD have shown no such correlation (Modigliani et al. 1990). The clinical relevance of MH in patients with IBD was first highlighted when treatment with azathioprine was shown to promote MH in CD (D’Haens et al. 1997). It was not until the era of TNFα-blocking therapy that MH has been widely recognized, and recent studies and
reviews have suggested that MH should be a therapeutic target in IBD (Frosli et al. 2007, Neurath and Travis 2012). Thus, no validated definition exists of what constitutes MH in IBD (Sandborn et al. 2002, D’Haens et al. 2007). The International Organization for the Study of Inflammatory Bowel Disease (IOIBD) proposed a definition of MH in UC as the absence of friability, blood, erosions, and ulcers in all visualized segments of gut mucosa (D’Haens et al. 2007). Similarly, for CD, the IOIBD tendered a consensus definition of MH that includes the absence of ulcers. However, this definition requires future validation (D’Haens et al. 2009). Simply stated, MH should imply the absence of ulcerations and erosions. Nevertheless, in CD, MH may be difficult to achieve.

6.2.4 TNFα-blocking therapy and mucosal healing

In the last 15 years, the advent of TNFα-blocking agents has offered new possibilities in the management of IBD. Data available from numerous studies have demonstrated that TNFα-blocking therapy can induce and maintain MH in IBD patients. In a study of 30 CD patients treated with a single dose of infliximab (IFX), a significant decrease in CDEIS in IFX group was seen, while patients in the placebo group experienced no endoscopic improvement (D’Haens et al. 1999b). In the ACCENT 1 (A Crohn’s Disease Clinical Trial Evaluating Infliximab in a New Long-Term Treatment Regimen) study (Hanauer et al. 2002), a randomized controlled trial evaluating the efficacy of IFX for the treatment of refractory active CD, an endoscopic substudy examining MH was performed in 99/573 randomized. Patients with induction therapy comprising three infusions of IFX 5 mg/kg achieved MH significantly more often than patients receiving only one infusion of IFX at baseline (Rutgeerts et al. 2004). Systematic maintenance therapy with IFX 5 mg/kg or 10 mg/kg every 8 weeks induced MH at week 54 in almost half of patients, and sustained MH in approximately 30% of patients. Episodic IFX therapy had a negligible impact on MH.

Similarly, in the SONIC (Study of Biologic and Immunomodulator Naïve Patients in Crohn’s Disease) trial (Colombel et al. 2010), induction therapy with IFX followed by scheduled maintenance treatment every 8 weeks resulted in MH in one-third of patients compared with 16% of patients receiving azathioprine alone at week 26 (Colombel et al. 2008). The EXTEND (extend the safety and efficacy of adalimumab through endoscopic healing) trial (Rutgeerts et al. 2012), a placebo-controlled endoscopy trial that evaluated the efficacy of ADA for the treatment of moderate-to-severe active ileocolonic CD. This trial was one of the first ones to use MH (absence of mucosal ulceration) as a primary endpoint. Induction therapy with ADA followed by scheduled maintenance therapy resulted in MH in 27.4% and 24.2% of patients at weeks 12 and 52, respectively, compared with 13.1% and 0% of patients receiving only induction treatment with ADA followed by placebo. The results of this study suggest that MH may be more difficult to achieve for patients with more severe ulcerations at baseline. The MUSIC (Endoscopic MUncoSal Improvement in Patients with Active Crohn’s Disease Treated with CZP) trial (Hebuterne et al. 2013) is an open-label study that assessed endoscopic improvement of disease in patients with active CD after
treatment with certolizumab pegol (CZP). Induction treatment followed by scheduled maintenance therapy resulted in endoscopic response (a decrease in CDEIS > 5) in about 50%, endoscopic remission (CDEIS < 5) in 27%, and MH (defined as the absence of ulcers) in only 8% of patients at week 54. Thus, the findings in this trial suggest that patients achieving MH after induction therapy (week 10) can maintain MH through week 54.

Anti-TNFα therapy may also lead to MH in UC patients. The ACT1 (Active Ulcerative Colitis Trial 1) and ACT2 (Active Ulcerative Colitis Trial 2) trials (Rutgeerts et al. 2005) investigated the role of IFX in refractory, moderate-to-severe active UC. MH was defined as a Mayo subscore ≤ 1 and was a secondary endpoint. Induction therapy with IFX resulted in MH in approximately 60% of patients at week 8. Scheduled maintenance therapy with IFX resulted in MH at week 30 in approximately 50% of patients. Data available only from the ACT1 trial showed MH in 46% of patients. ULTRA 1 and ULTRA 2 (Ulcerative colitis long-term remission and maintenance with adalimumab) trials, investigated the efficacy of adalimumab (ADA) in patients with moderate-to-severe active, refractory ulcerative colitis (Reinisch et al. 2011, Sandborn et al. 2012). As in ACT 1 and ACT 2 trials, MH was defined as a Mayo subscore ≤ 1 and set as a secondary endpoint. These studies demonstrated that induction treatment with resulted in MH in one-third of patients at week 8 regardless of whether or not they were previously treated IFX. The MH rate at week 52 was 25%. Most recently, studies (PURSUIT trials; Program of Ulcerative Colitis Research Studies Utilizing an Investigational Treatment) on the efficacy of induction and maintenance therapy with subcutaneous GLM in TNFα antagonist-naïve patients with moderate-to-severe UC have been published. The primary endpoint in the maintenance study was a continuous clinical response among GLM induction responders. As in previous studies, MH was a secondary endpoint and defined as a Mayo subscore ≤ 1. Approximately 40% of patients showed MH at both weeks 30 and 54 (Sandborn et al. 2014a and 2014b).

Differences in achieving MH in CD and UC can be explained by the fact that CD is a transmural disease, and therefore, the mucosa is more likely to heal in UC. Importantly, the optimal time for assessment of MH remain to some extent unresolved (Neurath and Travis 2012).

**6.2.5 Clinical impact of mucosal healing**

Schnitzler and coworkers (2009) showed in a large cohort study that in patients with scheduled IFX therapy MH was associated with fewer complications and better long-term outcome. Also patients achieving MH on maintenance therapy with IFX have had fewer hospitalizations and surgery and have attained a better quality of life (Lichtenstein et al 2002 and 2004). MH has also been demonstrated to lead to significantly higher steroid-free remission rates in patients with early-stage CD for as long as 4 years after start of therapy (Baert et al. 2010). A prospective study by the Norwegian IBSEN (Inflammatory Bowel South-Eastern Norway) group evaluated the course of UC in a population-based inception cohort and identified prognostic risk factors. They showed that patients who presented with MH
within one year of diagnosis had a significantly lower risk of colectomy (Solberg et al. 2009). The CHARM (Crohn’s disease trial of the fully Human antibody Adalimumab for Remission Maintenance) study, on the other hand, showed fewer hospitalizations and surgery in patients treated with ADA versus placebo (Colombel et al. 2007). The relative risk reduction was 57% at one year. However, this study lacks endoscopic data. In recent years, it has become more obvious that clinical remission alone is not an adequate treatment goal in IBD. A growing amount of evidence suggests that MH may lead to a better long-term outcome and should therefore be set as a comparative treatment goal.

**6.2.6 Deep remission**

A new therapeutic target in the scientific field is the concept of deep remission (DR). The definition of DR is still evolving. However, in CD, DR has been applied to patients on immunomodulators or TNFα-blocking therapy or both who have no clinical symptoms and no objective signs of inflammation (defined as Crohn’s disease activity index [CDAI < 150] and endoscopic remission) (Rutgeerts et al. 2012). In UC and IBDU, the definition of DR has not yet been well-defined.

**6.3 Histological activity**

Data on the prognostic relevance of histological healing are limited. However, several clinical studies indicate that effective medical therapy may contribute to MH (D’Haens et al. 1997, 1999a, 1999b, Geboes et al. 2005). However, whether histological healing can be achieved in CD with current medications and whether histological healing has any impact on the disease course remain unknown (Peyrin-Biroulet et al. 2011). On the other hand, histological healing seems to be relevant in UC, as microscopic inflammation without endoscopic lesions predicts relapse in patients who are in clinical and endoscopic remission (Bessissow et al. 2012). Moreover, persistence of microscopic inflammation has been stated to be an independent risk factor for the development of colorectal cancer in long-standing UC (Gupta et al. 2007). In a recent study, Lemmens and coworkers (2013) showed a nice correlation between the histological and endoscopic activity scores in UC, but pointed out also that important misclassifications exist for mild disease. Microscopy may detect more severe disease than endoscopically suspected. They also suggested that histological scoring should be used in addition to endoscopy when scoring disease activity for clinical trials (Lemmens et al. 2013). For these reasons, histological remission should be considered as the ultimate therapeutic goal in UC. Histologically, MH in UC is characterized by some features related to architectural damage and recovery, such as architectural crypt distortion (atrophy and branching), as well as epithelial regeneration, disappearance of basal plasmacytosis, and increased transmucosal cellularity. However, active inflammation is not usually present. Histological findings predicting relapse in patients with quiescent UC are basal plasmacytosis, increased transmucosal cellularity, high numbers of neutrophils and eosinophils, crypt abscesses, mucin depletion, and damage of the surface epithelium (Magro et al. 2013). No general
agreement exists among experts as to the value of microscopy in assessing CD activity, and therefore, histological remission cannot be a recommended endpoint in clinical trials (Sandborn et al. 2002). Several scoring systems for histological findings are available. The scoring systems most often used in clinical trials are shown in Tables 7 and 8 (D’Haens et al. 1998, 2007).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Epithelial damage</td>
<td>Normal</td>
</tr>
<tr>
<td>Architectural changes</td>
<td>Normal</td>
</tr>
<tr>
<td>Infiltration of mononuclear cells in lamina propria</td>
<td>Normal</td>
</tr>
<tr>
<td>Polymorphonuclear cells in lamina propria</td>
<td>Normal</td>
</tr>
<tr>
<td>Polymorphonuclear cells in epithelium</td>
<td>Normal</td>
</tr>
<tr>
<td>Presence of erosion and/or ulcers</td>
<td>No</td>
</tr>
<tr>
<td>Presence of granuloma</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 7. Histological findings in Crohn’s disease (D’Haens et al. 1998).
Table 8. Histological findings in ulcerative colitis (D’Haens et al. 2007).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Structural changes</td>
<td>Normal</td>
</tr>
<tr>
<td>Chronic inflammatory infiltrate</td>
<td>Normal</td>
</tr>
<tr>
<td>Infiltration of neutrophils in lamina propria</td>
<td>Normal</td>
</tr>
<tr>
<td>Neutrophils in epithelium</td>
<td>None</td>
</tr>
<tr>
<td>Presence of crypt destruction</td>
<td>None</td>
</tr>
<tr>
<td>Presence of erosion or ulceration</td>
<td>None</td>
</tr>
</tbody>
</table>

6.4 Blood tests

Anemia, leukocytosis, and thrombocytosis are common in the blood count of IBD patients with active disease; however, these findings are usually multifactorial. Anemia may develop due to an iron deficiency caused by hemorrhage or inflammation, leukocytosis may indicate extensive active inflammation or corticosteroid treatment, and thrombocytosis is usually a nonspecific response to inflammation, but may also occur during hemorrhage. ESR, which indirectly measures the acute-phase plasma protein concentration, is elevated during active inflammation as well as in any condition that elevates fibrinogen. In UC, the correlation between ESR and disease activity is good. However, ESR may be normal in proctitis and proctosigmoiditis (Sachar et al. 1986). In CD, the ESR appears to be a less accurate measure of disease activity. It appears to increase with increasing disease activity, but it correlates more with colonic disease and does not reflect the disease activity of the small bowel (Sachar et al. 1990, Desai et al. 2007). CRP, an acute-phase protein produced by the liver becomes elevated with tissue damage caused by inflammation, infection, or injury. The half-life of CRP is short; it rises rapidly and falls quickly (Desai et al. 2007). In CD,
CRP correlates with disease activity (Vermeire et al. 2004). For UC, the same trend is observed, although CRP is lower overall than in CD. More importantly, a high CRP value helps to identify UC patients at risk for colectomy (Travis et al. 1996). Low serum protein or albumin correlates inversely with clinical and endoscopic activity and is indicative for severe protein loss or malabsorption (Vermeire et al. 2006, Nikolaus and Schreiber 2007).

6.5 Cytokines

Tumor necrosis factor alpha (TNFα) is produced by activated macrophages and monocytes. In patients with active IBD, the serum concentration of TNFα is often temporarily increased, but not consistently elevated. Therefore, the value of serum concentration of TNFα as a surrogate marker of IBD activity is limited (Murch et al. 1991, Desai et al. 2007). The same conclusion applies to the measurement of TNFα concentration in stool (Braegger et al. 1992). IL-6, a cytokine produced by T-cells and macrophages, possesses both anti-inflammatory and pro-inflammatory effects. Elevated serum IL-6 concentrations are found in active CD, occasionally in UC (Mahida et al. 1992), and the level correlates closely with endoscopic findings, being higher in patients with inflammatory CD than with strictureing disease (Reinisch et al. 1999). Several other cytokines and cytokine receptors, such as IL-1RA, IL-1β, IL-2, IL-2Rα, TNF-R1, TNF-RII, IL-1RI, IL-1RII, IL-6, IL-6R, IL-8, IL-10, IL-15, IL-23, and IL-27, have been found to be elevated in IBD patients with active disease and may correlate with endoscopic and disease activity (Desai et al. 2007).

6.6 Stool tests

Active IBD inflammation produces activated leukocytes, mainly polymorphonuclear neutrophils, which infiltrate into the intestinal mucosa and can be detected in stool because of leukocyte shedding in the intestinal mucosa (Tibble et al. 2000a). Potential fecal biomarkers include fecal excretion of leukocytes, leukocyte products, and serum proteins (Desai et al 2007). Several S100-family proteins have been evaluated for their correlation with intestinal inflammation, fecal calprotectin being the most extensively studied. For detection of IBD inflammation, other useful neutrophil-derived biomarkers are lactoferrin (FL), S100A12 (calgranulin C), and polymorphonuclear neutrophil elastase (PMN-e).

6.6.1 Fecal calprotectin

Calprotectin is a calcium and zinc binding protein (also known as S100A8/S100A9) accounting for 60% of the cytosolic protein content of neutrophils (Dale et al. 1985). It is also expressed in activated macrophages and monocytes (Johe et al. 1997). Accumulation of neutrophils at the site of inflammation in the GI tract results in a release of calprotectin into feces. In the presence of calcium, calprotectin is stable and very resistant to bacterial degradation, and at room temperature it is stable in stool for up to 7 days (Røseth et al. 1992). Fecal calprotectin (FC) excretion has been shown to
correlate well with $^{111}$Indium test results in CD (Røseth et al. 1992, 1999, Tibble et al. 2000a), with histological and endoscopic assessment of disease activity in both CD and UC (Roseth et al. 1997, 2004 Sipponen et al. 2008a, 2008b, 2010a, 2010b, Schoepfer et al. 2010), and also with both disease extent and severity in UC (D’Haens et al. 2012). As FC can differentiate between active and inactive IBD, it can also differentiate between IBD and irritable bowel syndrome (IBS) (Tibble et al. 2002, Schoepfer et al. 2008). Gisbert and coworkers (2009a) reported a sensitivity of 83% and a specificity of 84% for FC in distinguishing between organic and nonorganic disease. Furthermore, FC is known to be specific for inflammation, but is not disease-specific. It may increase also in other GI inflammatory conditions where neutrophils are present such as colorectal carcinoma, diverticulosis, GI infections, NSAID enteropathy, microscopic colitis, or even untreated celiac disease (Roseth et al. 1993, Tibble et al. 1999, Limburg et al. 2000, Berni Canani et al. 2004, Sipponen 2013). For the diagnosis of IBD, FC seems to be superior to other serological markers, including CRP, ESR, ANCA, and ASCA (Tibble et al. 2000a, 2001, Schoepfer et al. 2007, 2008, Langhorst et al. 2008). The optimal cut-off value for distinguishing between inactive and active IBD remains somewhat unsettled. According to the manufacturer, an FC level < 50 μg/g is normal. However, in clinical practice a cut-off value < 100 μg/g is frequently used for FC to separate inactive and active disease (von Roon et al. 2007, Sipponen et al. 2008b), and several other cut-off levels have been described in clinical trials (Kornikoff et al. 2006). In active inflammation of the gut, FC may rise to tens of thousands of micrograms/gram; however, considerable intraindividual variation in FC concentration exists (Moum et al. 2010), and even patients without inflammation or neoplasm show day-to-day variation in FC release (Huseby et al. 2011). Interestingly, FC has a significant positive relationship with increasing age, physical inactivity, and obesity and an inverse relationship with vegetable consumption and fiber intake (Poullis et al. 2004). Moreover, studies of FC in assessing intestinal inflammation are scarce. A UK study demonstrated that FC may have a role in assessing small bowel CD, often beyond the reach of conventional colonoscopy (Dolwani et al. 2004). A FC level > 60 μg/g was shown to predict abnormal barium follow-through results, and the negative predictive value of a single FC level < 60 μg/g was 100%. Somewhat contrary to this, Sipponen and coworkers (2012) found that FC had a low utility for predicting the presence of small bowel CD on WCE, with a sensitivity of 59% and a specificity of 71% using a cut-off of 50 μg/g.

FC can be quantified from feces by several different enzyme-linked immunosorbent assays (ELISAs). In 1992, Roseth and coworkers developed a method for extraction of calprotectin from feces and quantification by an enzyme-linked immunoassay. The method was improved in 2000, when its units were changed from mg/l to μg/g (Tibble et al. 2000a). Recently, rapid semiquantitative and quantitative tests have been developed and validated for identifying patients with GI inflammation. These tests have been shown to detect endoscopic activity and postoperative recurrence as accurately as the ELISA tests (Lobaton et al. 2013). The use of these rapid tests in clinical practice has been limited, and ELISA test is the most widely used for measuring FC concentration. FC allows a noninvasive monitoring of disease
activity. However, the ELISA test is time-consuming and requires a well-trained laboratory personnel, and collection of multiple samples to be cost-effective.

The clinical course of these chronic, remitting, and relapsing conditions is often unpredictable, and the assessment of the severity of IBD and the monitoring of disease activity are important issues, in both CD and UC. Growing evidence suggests that MH reflects controlled IBD activity with a more favorable course (Arnott et al. 2002, Rutgeerts et al. 2007, Van Assche et al. 2010b). Low FC level is considered a valuable surrogate marker for MH, with high levels indicating a risk of relapse. A growing number of studies have demonstrated the ability of a single FC concentration to predict clinical relapse in IBD patients with clinically quiescent disease. A list of these studies is presented in Table 9. According to these studies, it seems that especially in colonic CD and UC, a single elevated FC concentration in clinically inactive IBD patients predicts a clinical relapse within one year. More recently, a meta-analysis of six prospective studies showed a pooled sensitivity of 78% (95% CI 72–83%) and a specificity of 73% (95% CI 68–77%) for FC in predicting IBD relapse (Mao et al. 2012). The study of infliximab discontinuation in Crohn’s disease patients in stable remission on combined therapy with immunosuppressors (STORI trial), the largest study exploring the duration of remission in CD after discontinuation of TNFα-blocking therapy, showed that FC concentration >300 μg/g at inclusion was an independent factor associated with disease relapse and concluded that an elevated FC concentration predicts the risk of IBD relapse (Louis et al. 2012).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of patients and diagnosis</th>
<th>FC cut-off (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibble et al. 2000b</td>
<td>37; UC</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Tibble et al. 2000b</td>
<td>43; CD</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Costa et al. 2005</td>
<td>41; UC</td>
<td>&gt; 150</td>
</tr>
<tr>
<td>Costa et al. 2005</td>
<td>38; CD</td>
<td>&gt; 150</td>
</tr>
<tr>
<td>D’Inca et al. 2008</td>
<td>97; UC</td>
<td>&gt; 130</td>
</tr>
<tr>
<td>D’Inca et al. 2008</td>
<td>65; CD</td>
<td>&gt; 130</td>
</tr>
<tr>
<td>Walkiewicz et al. 2008</td>
<td>32; CD</td>
<td>&gt; 130</td>
</tr>
<tr>
<td>Diamanti et al. 2008</td>
<td>73; UC/CD</td>
<td>&gt; 275</td>
</tr>
<tr>
<td>Kallel et al. 2010</td>
<td>53; CD</td>
<td>&gt; 150</td>
</tr>
<tr>
<td>Sipponen et al. 2010b</td>
<td>72; UC/CD</td>
<td>&gt; 340</td>
</tr>
</tbody>
</table>

FC = fecal calprotectin; UC = ulcerative colitis; CD =Crohn’s disease
6.6.2 Lactoferrin and S100A12

Lactoferrin (FL) and S100A12 are the other two potential fecal surrogate markers for monitoring IBD patients in clinical practice. FL is an iron-binding glycoprotein secreted by most mucosal membranes that interact directly with external pathogens. FL is a major component of the secondary granules of polymorphonuclear neutrophils, which are a primary component of the acute inflammatory response (Kayazawa et al. 2002), and can be detected in mammalian milk, saliva, semen, tears, bile, and mucosal secretions. Like calprotectin, FL is a nonspecific marker of intestinal inflammation and can be detected in several conditions besides IBD, such as infective diarrhea, colon neoplasia, and acute radiation-induced proctitis (Uchida et al. 1994, Dai et al. 2007, Hille et al. 2008). Many previous studies have indicated the potential usefulness of measuring FL in patients with IBD (Fine et al. 1998, Tibble and Bjarnason 2001, Kane et al. 2003). During inflammation FL levels quickly increase (Desai et al. 2007), and correlations between FL and endoscopic and/or histological activity in IBD patients have been detected (Langhorst et al. 2008, Sipponen et al. 2008a, 2008b). FL has also been shown to predict relapse in IBD patients, as Gisbert and coworkers (2009a) reported a sensitivity of 62% and a specificity of 65% in predicting relapse in clinical remission.

Neutrophil-derived protein S100A12, also known as calgranulin C, has been suggested as a fecal marker of intestinal inflammation. It is a member of the damage-associated molecular pattern proteins and is released from neutrophils or monocytes under conditions of cell stress. S100A12 is remarkably resistant to degradation by fecal bacteria, and fecal specimens show stability for at least 7 days at room temperature (de Jong et al. 2006). A growing amount of data indicates S100A12 to be a suitable marker of IBD, and several studies have demonstrated a correlation between mucosal inflammation and S100A12 levels in blood (Leach et al. 2007, Manolakis et al. 2010) and feces (de Jong et al. 2006, Kaiser et al. 2007) of patients with IBD. Fecal S100A12 is a promising marker of intestinal inflammation, with specificity superior to FC in predicting IBD relapse. Higher baseline levels of fecal S100A12 are significantly associated with disease relapse during the course of the disease, and fecal S100A12 levels are already increased up to 6 months before clinical relapse in both CD and UC (Däbritz et al. 2013).
7 Treatments

Treatment goals in IBD include induction and maintenance of remission, mucosal healing, avoidance of hospitalization and surgery, minimizing the risk of cancer, and optimizing the quality of life. When making decisions on medical therapy, severity of the disease (mild, moderate, severe), localization (CD: ileal, ileocolonic, colonic, or other; UC: extensive, left-sided, or proctitis), and behaviour in CD (inflammatory, strictureing, or fistulating) should be taken into consideration. Factors that contribute to the individual management of IBD patients include extraintestinal manifestation of IBD and comorbidities, patient preferences and benefits, intolerance to medications, and also such life situations as schooling, work, and family life that may particularly influence the timing and choice of some treatments. In some CD patients with mild disease, no medication can be an option. As smoking cessation in CD is associated with a more benign disease course and better treatment outcomes, smoking cessation advice should be offered to all patients (Cosnes et al. 2001). No curative drug is available.

7.1 Medical therapy

7.1.1 Conventional therapy

Corticosteroids have been used in active IBD since 1955, when Truelove and Witt published their study on the efficacy of corticosteroid treatment in active UC. Most IBD patients respond to the first-line corticosteroid therapy, but more than half will develop a steroid-dependent or steroid-resistant disease already at one year (Munkholm et al. 1994). MH can be achieved only in about one-third of IBD patients. In mildly active ileocolic CD, enteric-coated budesonide is a treatment option. It has fewer unwanted systemic side-effects than conventional corticosteroid therapy, because of its rapid hepatic conversion to metabolites. Even so, severely active or extensive ileocecal or ileal CD should be treated with systemic corticosteroids (Dignass et al. 2010). Corticosteroids play no role in maintaining remission.

5-aminosalicylic acid (5-ASA) drugs are the mainstay of therapy for mild to moderate UC. The standard first-line induction therapy for distal UC involves topical 5-ASA drugs, which have a more rapid effect than oral therapy. A combination of oral and topical 5-ASA appears to provide a further benefit. Sulfasalazine is as effective as mesalazine, but has more side-effects and adverse events than mesalazine (Dignass et al. 2012a). In UC, oral 5-ASA treatment can induce and maintain remission in minority of patients, while in CD the effect of 5-ASA on induction or maintenance of remission is limited (Hanauer and Strömberg 2004).

Immunomodulative therapy for IBD consists of thiopurines (azathioprine and 6-mercaptopurine) and metotrexate. Thiopurines have been shown to be effective and superior to 5-ASA in inducing and maintaining remission in steroid-dependent or steroid-refractory UC (Ardizzone et al. 2006) and can successfully prevent relapse in patients resistant to 5-ASA therapy. Immunomodulative therapy is also recommended for postoperative
prophylaxis of complex CD and for extensive small bowel disease (Dignass et al. 2010). In active CD, thiopurines induce and maintain remission and lead to MH (D’Haens et al. 1997, 1999a, Prefontaine et al. 2009, 2010). In patients requiring rapid induction of remission, thiopurines are unsuitable because of their slow onset of action (Dignass et al. 2010, 2012a). Methotrexate is more effective than placebo in inducing and maintaining clinical and endoscopic remission in active CD during intramuscular therapy (Feagan et al. 2000).

Due to the role of the microbiome in IBD pathogenesis, interest in the use of probiotics in IBD has grown. Promising results have emerged for E. coli Nissle in inactive UC to induce clinical remission. Also the probiotic mixture VSL#3 has been shown to be more effective than placebo in inducing clinical response in active UC (Dignass et al. 2012a). No evidence to support the use of probiotics in CD is available (Dignass et al. 2010).

Antibiotics, such as ciprofloxacin and metronidazole, are appropriate in treating infectious complications, perianal disease, and bacterial overgrowth (Dignass et al. 2010, 2012a). Table 10 shows the MH rates in different treatment groups.

<table>
<thead>
<tr>
<th>Table 10. Conventional medical therapy and mucosal healing rates.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical therapy</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Corticosteroids:</td>
</tr>
<tr>
<td>prednisolone</td>
</tr>
<tr>
<td>budesonide</td>
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<tr>
<td>Mesalazine</td>
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<td></td>
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<td></td>
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<tr>
<td>Thiopurines:</td>
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<tr>
<td>azathioprine</td>
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<td></td>
</tr>
<tr>
<td>Methotrexate</td>
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</table>
7.1.2 TNFα-blocking agents

The pro-inflammatory cytokine TNFα plays an important role in the pathogenesis of IBD. Anti-TNFs interrupt the inflammatory process by neutralizing this cytokine by several mechanisms. TNFα-blocking agents are biological drugs, thus produced by biological processes.

7.1.2.1 Indications for TNFα-blocking agents in IBD

Anti-TNF antibodies have been found to be an effective treatment for moderate to severe CD and UC. However, the optimal timing of anti-TNFα therapy may be difficult to determine. A therapy started too late will be unable to impact the surgical rate. CD patients with extensive or perianal disease, abdominal or complex perianal fistulizing lesions, or multiple deep ulcers and UC patients with severe extensive colitis with no response to steroids, may benefit the most from the early onset of anti-TNF therapy.

IFX is an intravenously administered murine-divided chimeric IgG1 anti-TNF monoclonal antibody with potent anti-inflammatory effects, possibly dependent on apoptosis of inflammatory cells. Induction therapy with IFX at a dose of 5 mg/kg or 10 mg/kg at 0, 2, and 6 weeks followed by maintenance therapy every 8 weeks can be considered in outpatients with steroid-dependent or -refractory disease and should be used in patients failing azathioprine or 6-mercaptopurine monotherapy in both CD and UC. In severe or fulminant UC, IFX is superior to ADA (Dignass et al. 2012a). Patients with active fistulizing CD despite treatment with antibiotics and AZA or 6-MP can be treated with IFX (at 0, 2, and 6 weeks for induction, and then every 8 weeks for maintenance) as monotherapy or combined with other immunomodulating agents (Present et al. 1999, Sands et al. 2004, Colombel et al. 2010).

ADA is a subcutaneously (SC) administered, recombinant, fully human, immunoglobulin G1 monoclonal antibody that binds with high affinity and specificity to human TNF, but not to lymphotoxin, and modulates biologic responses induced or regulated by TNF (Sandborn et al. 2012). ADA is given subcutaneously 160 mg at week 0 and 80 mg at week 2, and then 40 mg every other week beginning at week 4 or if necessary weekly for maintenance in both CD and UC (Hanauer et al. 2006, Colombel et al. 2007, Sandborn et al. 2007b, 2012a). ADA has also been demonstrated to be effective in fistulizing CD, and in patients who have developed antibodies against IFX (Rutgeerst et al. 2012).

GLM is a subcutaneously (SC) administered, fully human, anti-TNF antibody, approved for the treatment of UC in 2013. GLM seem to have similar efficacy in UC as ADA, however, its advantages compared with IFX and ADA are its low immunogenicity, weight-based, and once-monthly administration (Sandborn et al. 2014a, 2014b). No trials on the efficacy of GLM in CD exist.
Biosimilars of antibodies to TNFα are becoming important in the treatment of IBD. Experience with simple peptide biosimilars, such as epoetins and growth factors, has generally been positive, with these biosimilars having similar efficacy and safety as the original products. So far, only an IFX biosimilar is available. However, immunogenicity remains a major concern. Future studies will address concerns on biosimilars in IBD, including their efficacy, safety, immunogenicity, switching, and interchangeability.

Other immunomodulatory agents, such as cyclosporine, tacrolimus mycophenolate mofetil, thalidomide, and thioguanine, have also been advocated in the management of IBD (Dignass et al. 2010, 2012a). These treatments will not be discussed in any detail in this review. A strong need exists for more effective medication, especially in CD, where surgery is not a curative treatment option. CZP is a pegylated, subcutaneously administered humanized anti-TNF Fab (fragment antigen binding) -antibody fragment with proven clinical efficacy despite the lack of pro-apoptotic effects (Schreiber et al. 2005). Earlier studies have shown CZP to have similar efficacy as other TNFα-agents especially in anti-TNF-naïve CD patients and those failing IFX therapy (Sandborn et al. 2007c, 2010, Schreiber et al. 2007). Thus, CZP has no indications in Finland. Vedolizumab, a fully humanized α4β7 integrin antibody, is more effective than placebo in inducing and maintaining remission in both CD and UC (Feagan et al. 2013, Sandborn et al. 2013b). After natalizumab, a α4-integrin, resulted in reactivation of human papillomavirus, leading to a progressive multifocal leukoencephalopathy (PLE), some concerns have been raised. As vedolizumab modulates only the gut, not the brain, no cases of PLE have emerged (Soler et al. 2009). The primary data on ustekinumab, IL-12 and IL-23 inhibitors, suggest that it might be effective in particular in CD patients failing TNFα-blocking therapy (Sandborn et al. 2012b). Other IL-12 and IL-23 inhibitors, such as apilimod and briakinumab, are still in phase II trials. Tofacitinib, an inhibitor of Janus kinases 1, 2, and 3, has shown promise in UC treatment, and trials on tofacitinib have advanced to phase III (Sandborn et al. 2012c).

### 7.1.2.2. Efficacy and safety of TNFα-blocking therapy

Data on efficacy, treatment outcomes, and safety of TNFα-blocking therapy in IBD are extensive.

IFX has been demonstrated to be efficacious for the treatment of refractory luminal and fistulizing CD in several studies. In the short term, up to 80% of patients experience a rapid improvement of their symptoms and up to 50% have a complete remission (Present et al. 1999, Hanauer et al. 2002, Rutgeerts et al. 2004a). With scheduled maintenance treatment, IFX not only maintains disease improvement, but also induces clinical improvement in about two-thirds of patients and MH in nearly half of the treated patients (Present et al. 1999, Hanauer et al. 2002, Sands et al. 2004, Rutgeerts et al. 2006). Data from the ACCENT I and II (A Crohn’s Disease Clinical Trial Evaluating Infliximab in a New Long-term Treatment Regimen in Patients With Fistulizing Crohn’s Disease) studies also show that scheduled treatment with IFX decreases the rate of hospitalizations and surgical interventions.
(Hanauer et al. 2002, Sands et al. 2004). Patients achieving MH on maintenance therapy with IFX experience fewer hospitalizations and surgeries, have a better quality of life, and also have a better long-term outcome (Lichtenstein et al. 2002, 2004, Schnitzler et al. 2009). In UC, data on long-term outcome of TNFα-blocking therapy are based mainly on the ACT 1 trial. Scheduled maintenance therapy with IFX 5 mg/kg resulted in clinical remission in about 35% and MH in about 46% of patients by week 54 (Rutgeerts et al. 2005). Recently published data (the UC SUCCESS trial; the study of efficacy and safety of infliximab and azathioprine therapy alone or in combination for ulcerative colitis) revealed that combination treatment with IFX and azathioprine in UC patients resulted in higher remission rates than IFX or azathioprine monotherapy (Panaccione et al. 2014). In addition, a detectable trough in serum IFX predicts clinical remission, endoscopic improvement, and a lower risk of colectomy in UC patients (Seow et al. 2010).

ADA has been effective in inducing clinical response and remission in both TNF-naïve and IFX-failure patients (Hanauer et al. 2006, Sandborn et al. 2007b, 2009). The CHARM I and II studies demonstrated a steroid-free clinical remission rate at week 56 for 29% of patients, and also fewer hospitalizations and surgery (Colombel et al. 2007, Feagan et al. 2008). Unfortunately, these studies lack endoscopic data. The SONIC study showed a steroid-free clinical remission rate at week 26 of 44% (Colombel et al. 2010). However, according to the EXTEND trial, DR, defined as a combination of clinical remission (CDAI <150) and complete MH, was achievable in some 19% of patients by week 52 (Rutgeerts et al. 2012). Importantly, in a recent study of patients with moderate to severe ileocolonic CD who received ADA induction and maintenance therapy, patients achieving deep remission appeared to have better one-year outcomes than those not achieving DR (Colombel et al. 2014). In the ULTRA I and II trials, ADA-treated patients achieved clinical remission significantly more often at week 8 and week 52 (Reinisch et al. 2011, Sandborn et al. 2012a). ADA treatment is associated with a positive benefit/risk balance in the overall population of patients with moderate-to-severe active UC in ULTRA 2; moreover, early response was predictive of a positive outcome at one year (Sandborn et al. 2013a).

Based on the outcomes of the PURSUIT-SC induction study, induction treatment with GLM was concluded to be effective and safe for patients with moderate to severe active UC. Even so, the clinical remission and MH rates at week 6 are fairly low. The PURSUIT maintenance study demonstrated continuous clinical response (primary endpoint), clinical remission, and MH (secondary endpoints) at week 30 and week 54 among GLM induction responders significantly more often in patients receiving active treatment with 50 mg and 100 mg of GLM versus placebo (Sandborn et al. 2014b). Clinical response, clinical remission and MH rates of TNFα-blocking therapy are summarized in Tables 11 and 12.
Table 11. Endpoints of induction and maintenance with TNFz-blocking therapy in CD.

<table>
<thead>
<tr>
<th>Study</th>
<th>Reference</th>
<th>Anti-TNF used</th>
<th>Clinical response rate</th>
<th>Clinical remission rate</th>
<th>Mucosal healing rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACCENT 1</td>
<td>Hanauer et al. 2002</td>
<td>IFX</td>
<td>At week 30</td>
<td></td>
<td>39% (5 mg/kg)</td>
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<td></td>
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<td></td>
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<td></td>
<td>45% (10 mg/kg)</td>
</tr>
<tr>
<td>Endoscopic substudy of</td>
<td>Rutgeerts et al. 2006</td>
<td>IFX</td>
<td>At week 54</td>
<td>46% (5 mg/kg)</td>
<td>55% (10 mg/kg)</td>
</tr>
<tr>
<td>ACCENT 1</td>
<td></td>
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<td></td>
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<tr>
<td>EXTEND</td>
<td>Rutgeerts et al. 2012</td>
<td>ADA</td>
<td>At week 12; 47%</td>
<td>At week 52; 27%</td>
<td>At week 12; 27%</td>
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<tr>
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<tr>
<td>CLASSIC I</td>
<td>Hanauer et al. 2006</td>
<td>ADA</td>
<td>At week 4</td>
<td>18% (40/20 mg)</td>
<td>24% (80/40 mg)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36% (160/80 mg)</td>
<td></td>
</tr>
<tr>
<td>CHARM</td>
<td>Colombel et al. 2007</td>
<td>ADA</td>
<td>At week 26</td>
<td>89%/82%</td>
<td>At week 26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40%/47%</td>
<td>(40 mg eow/e w)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36%/41%</td>
</tr>
<tr>
<td>CHARM (open label</td>
<td>Panaccione et al. 2010</td>
<td>ADA</td>
<td>At 2 years</td>
<td>42% (40 mg eow)</td>
<td>At 2 years</td>
</tr>
<tr>
<td>extension)</td>
<td></td>
<td></td>
<td></td>
<td>50% (40 mg ew)</td>
<td>67%</td>
</tr>
<tr>
<td>CHARM (open label</td>
<td>Lofus et al. 2009</td>
<td>ADA</td>
<td>At 2 years</td>
<td>65%</td>
<td>At 3 years</td>
</tr>
<tr>
<td>extension)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>69%</td>
</tr>
<tr>
<td>SONIC</td>
<td>Colombel et al. 2010</td>
<td>ADA</td>
<td>At week 26; 44%</td>
<td></td>
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</tr>
</tbody>
</table>

ADA = adalimumab; IFX = infliximab; eow = every other week; ew = every week
<table>
<thead>
<tr>
<th>Study</th>
<th>Reference</th>
<th>Anti-TNF used</th>
<th>Clinical response rate</th>
<th>Clinical remission rate</th>
<th>Mucosal healing rate</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>At week 8</td>
<td>At week 8</td>
<td>At week 8</td>
</tr>
<tr>
<td>ACT I</td>
<td>Rutgeerts et al. 2005</td>
<td>IFX</td>
<td>65% (5 mg/kg)</td>
<td>39% (5 mg/kg)</td>
<td>62% (5 mg/kg)</td>
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<td></td>
<td></td>
<td></td>
<td>69% (10 mg/kg)</td>
<td>32% (10 mg/kg)</td>
<td>57% (10 mg/kg)</td>
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<td></td>
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<td></td>
<td>At week 30</td>
<td>At week 30</td>
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<td></td>
<td></td>
<td></td>
<td>47% (5 mg/kg)</td>
<td>34% (5 mg/kg)</td>
<td>50% (5 mg/kg)</td>
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<td></td>
<td></td>
<td></td>
<td>60% (10 mg/kg)</td>
<td>35% (10 mg/kg)</td>
<td>49% (10 mg/kg)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>At week 54</td>
<td>At week 54</td>
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<td></td>
<td></td>
<td></td>
<td>46% (5 mg/kg)</td>
<td>35% (5 mg/kg)</td>
<td>46% (5 mg/kg)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>44% (10 mg/kg)</td>
<td>34% (10 mg/kg)</td>
<td>47% (10 mg/kg)</td>
</tr>
<tr>
<td>ACT II</td>
<td>Rutgeerts et al. 2005</td>
<td>IFX</td>
<td>65% (5 mg/kg)</td>
<td>34% (5 mg/kg)</td>
<td>60% (5 mg/kg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>69% (10 mg/kg)</td>
<td>27% (10 mg/kg)</td>
<td>62% (10 mg/kg)</td>
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<td></td>
<td>At week 30</td>
<td>At week 30</td>
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<td></td>
<td></td>
<td></td>
<td>47% (5 mg/kg)</td>
<td>26% (5 mg/kg)</td>
<td>46% (5 mg/kg)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>60% (10 mg/kg)</td>
<td>36% (10 mg/kg)</td>
<td>57% (10 mg/kg)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>At week 54</td>
<td>At week 54</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>52% (80/40 mg)</td>
<td>10% (80/40 mg)</td>
<td>38% (80/40 mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>55% (160/80 mg)</td>
<td>19% (160/80 mg)</td>
<td>47% (160/80 mg)</td>
</tr>
<tr>
<td>ULTRA 1</td>
<td>Reinisch et al. 2011</td>
<td>ADA</td>
<td>At week 52</td>
<td>At week 52</td>
<td>At week 52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30%</td>
<td>17%</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>At week 6</td>
<td>At week 6</td>
<td>At week 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51% (200/100 mg)</td>
<td>18% (200/100 mg)</td>
<td>42% (200/100 mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>55% (400/200 mg)</td>
<td>18% (400/200 mg)</td>
<td>45% (400/200 mg)</td>
</tr>
<tr>
<td>PURSUIT SC</td>
<td>Sandborn et al. 2014a</td>
<td>GLM</td>
<td>At weeks 30 and 54</td>
<td>At weeks 30 and 54</td>
<td>At weeks 30 and 54</td>
</tr>
<tr>
<td>Induction</td>
<td></td>
<td></td>
<td>47% (50 mg)</td>
<td>23% (50 mg)</td>
<td>42% (50 mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50% (100 mg)</td>
<td>28% (100 mg)</td>
<td>42% (100 mg)</td>
</tr>
</tbody>
</table>

ADA = adalimumab; GLM = golimumab; IFX = infliximab
Unfortunately, a significant proportion of IBD patients, especially UC patients, do not respond to TNFα-induction therapy (primary nonresponders) or require dose escalation due to loss of response over time (secondary nonresponders). Determination of serum trough levels (TL), anti-infliximab antibodies (ATI), and anti-adalimumab antibodies (ATA) has been proposed as a method to optimize anti-TNF therapy. A detectable trough level has been demonstrated to be a strong predictor for remission in both CD and UC (Maser et al. 2006, Seow et al. 2010). Development of ATI is early in the course of treatment and can cause loss of drug efficacy and adverse reactions such as infusion reactions. Episodic treatment with IFX is associated with a higher likelihood of developing ATI (30–61%) than maintenance treatment (7–10%) or treatment with immunomodulators (10–43%) (Hanauer et al. 2004, Baert et al. 2003). In some cases, adverse events require discontinuation of therapy, and in these cases switching to another anti-TNF agent is generally recommended.

Treatment with anti-TNF agents seems to be relatively safe (Blonski and Lichtenstein 2007, Hoentjen et al. 2009, Lees et al. 2009, Lichtenstein et al. 2012, Burmester et al. 2013). The most common side-effects are acute infusion reactions (<4–23%) or injection-site reactions (13.3–29.3%) and infections (20.8–49.4%). Severe infections are significantly more uncommon (3.0–9.9%). Other reported side-effects, such as delayed hypersensitivity reactions most typically with arthralgia and muscle ache, serum sickness-like reactions, drug-induced lupus, demyelinating complications, other autoimmune diseases, and malignancies, are relatively rare. Acute infusion reactions are seldom serious and can be treated with prophylactic antihistamines or a single dose of hydrocortisone. After a period exceeding 4 months of no drug treatment, patients are more likely to develop antibodies against anti-TNF agents and therefore to suffer from infusion reactions. Prophylactic medication should be considered for these patients. Current data from clinical trials do not indicate that patients treated with IFX have an increased risk of developing lymphoma (Rutgeerts et al. 2004b). However, a recent meta-analysis of 6 studies found that patients treated with azathioprine or 6-mercaptopurine had a more than 4-fold increase in the risk of lymphoma, and therefore the association between IFX and lymphoma is remains obscure (Kandiel et al. 2005). Moreover, the TREAT (Crohn’s Therapy, Resource, Evaluation, and Assessment Tool) registry demonstrated no significant increase for malignancies in patients treated with IFX (Lichtenstein et al. 2012). Similar results have also been reported with other anti-TNF agents. Most recently, Osterman and coworkers (2014) demonstrated that the incidence of malignancy with ADA monotherapy in CD patients is equal to that of the general population. However, an aggressive hepatoplenic T-cell lymphoma has been associated with the use of anti-TNF agents (Shale et al. 2008). It is still unclear whether the anti-TNF agents used, the thiopurine therapy, the concomitant immunosuppressive therapy, or the underlying disease, separately or in combination, are risk factors for the development of these lymphomas. Despite improved disease control with combination therapy, it is conceivable that high levels of immunosuppression could increase the risk of complications, including death. Moreover, a concomitant immunosuppression appears to be an
important risk factor for infections during TNFα-blocking therapy. Serious opportunistic infections, such as tuberculosis and histoplasmosis, occur at a higher incidence when concomitant immunosuppression is employed. In any case, the absolute incidence of opportunistic infections and mortality are low (Toruner et al. 2008, Colombel et al. 2004). Side-effects associated with TNFα-blocking therapy are summarized in Table 13.

Table 13. Most typical side-effects associated with anti-TNF therapy.

<table>
<thead>
<tr>
<th>Side-effects</th>
<th>Example</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infections</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>Keane et al. 2001</td>
<td></td>
</tr>
<tr>
<td>Histoplasmosis</td>
<td>Hanauer et al. 2002</td>
<td></td>
</tr>
<tr>
<td>Candidias</td>
<td>Tsiodras et al. 2008</td>
<td></td>
</tr>
<tr>
<td>Aspergillosis</td>
<td>Toruner et al. 2008</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Burmester et al. 2012</td>
</tr>
<tr>
<td><strong>Antibody formation and autoimmunity</strong></td>
<td>Antibodies to infliximab</td>
<td>Sand et al. 2002</td>
</tr>
<tr>
<td></td>
<td>Antibodies to adalimumab</td>
<td>Baert et al. 2003</td>
</tr>
<tr>
<td></td>
<td>Antinuclear antibodies</td>
<td>Vermeire et al. 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rutgeerts et al. 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sandborn et al. 2007b</td>
</tr>
<tr>
<td><strong>Infusion reaction /injection-site reactions</strong></td>
<td>Anaphylaxis</td>
<td>Cheifetz et al. 2003</td>
</tr>
<tr>
<td></td>
<td>Serum sickness-like disease</td>
<td>Hanauer et al. 2002 and 2006</td>
</tr>
<tr>
<td></td>
<td>Delayed-type hypersensitivity</td>
<td>Colombel et al. 2007</td>
</tr>
<tr>
<td></td>
<td>Rash</td>
<td>Sandborn et al. 2007b</td>
</tr>
<tr>
<td><strong>Demyelization</strong></td>
<td>Demyelinating neuropathy</td>
<td>Mohan et al. 2001</td>
</tr>
<tr>
<td></td>
<td>Guillain-Barre syndrome</td>
<td>Shin et al. 2006</td>
</tr>
<tr>
<td><strong>Malignancies</strong></td>
<td>Lymphomas (RA)</td>
<td>Hanauer et al. 2002</td>
</tr>
<tr>
<td></td>
<td>Hepatosplenic T-cell lymphoma</td>
<td>Bongartz et al. 2006</td>
</tr>
<tr>
<td></td>
<td>Non-melanoma skin cancer</td>
<td>Shale et al. 2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Burmester et al. 2012</td>
</tr>
<tr>
<td><strong>Abnormal liver function tests</strong></td>
<td>Hepatitis</td>
<td>Menghini et al. 2001</td>
</tr>
<tr>
<td></td>
<td>Cholestatic diseases</td>
<td>Moun et al. 2007</td>
</tr>
<tr>
<td><strong>Dermatological symptoms</strong></td>
<td>Psoriatic dermatitis</td>
<td>Fidder et al. 2007</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>Joint pain and myalgias</td>
<td>Sandborn et al. 2004</td>
</tr>
<tr>
<td></td>
<td>Parethesias, weakness</td>
<td>Rutgeerts et al. 2005</td>
</tr>
<tr>
<td></td>
<td>Headache, dizziness</td>
<td>Schreiber et al. 2005</td>
</tr>
<tr>
<td></td>
<td>Nausea and vomiting</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chest pain, dyspnea, and cough</td>
<td></td>
</tr>
</tbody>
</table>

RA = Rheumatoid arthritis
7.1.2.3 Discontinuation of TNFα-blockling therapy

Despite the favorable effects of TNFα-blocking therapy, the fear of side-effects, intolerance, or desired pregnancy may necessitate discontinuation of TNFα-blocking therapy. Also, quite disappointingly, a recent systematic survey and meta-analysis demonstrated no reduction in the need for surgery in IBD patients on TNFα-blocking therapy (Ehteshami-Afshar et al. 2011). Another important basis for stopping TNFα-blocking therapy is economical. Sensitivity analyses have highlighted the fact that cost-effectiveness is to some extent influenced by the treatment duration, and that treatments beyond 4 years not being cost-effective (Bodger et al. 2009).

Data available on treatment withdrawal are limited. The observational, prospective STORI trial of 115 CD patients showed that stopping IFX while on combination therapy with immunomodulators leads to clinical recurrence in approximately 50% of cases within 1 or 2 years, with a median time to relapse of 16.2 months (Louis et al. 2012). The STORI trial identified male gender, absence of previous surgical resection, steroid treatment in the preceding 6-12 months, CDEIS > 0, hemoglobin < 145 g/L, white blood count > 6.0x10⁹/L, CRP level > 5.0 mg/L, FC > 300 µg/g, and high drug trough level as predictors of relapse after the discontinuation of IFX. In a longitudinal cohort study, Waugh and coworkers (2010) enrolled 48 patients with CD in full steroid-free clinical remission for at least 6 months who had discontinued IFX for reasons other than loss of response and monitored these patients for up to 7 years. Up to 50% of these patients relapsed within 477 days after IFX discontinuation. More importantly, 35% of patients remained well and without clinical relapse. No risk factors for relapse were indentified in this study. An observational cohort study explored the long-term clinical benefit of IFX in 614 consecutive patients with CD, 577 having an initial response to IFX. During follow-up, approximately one-third had to stop the medication because of side-effects, loss of response, or other reasons. Altogether 110 patients in clinical remission stopped IFX and remission was maintained for a median of 47.3 months (Schnitzler et al. 2009). In a follow-up study “step up versus step down” (D’Haens et al. 2008), 49 patients with early-stage CD who underwent ileocolonoscopy after 2 years of therapy were followed up for an additional 2 years. Of 24 patients with complete MH, 17 maintained remission without steroids and of these 17 patients, 15 maintained remission without IFX and three were in remission without even an immunomodulator (Baert et al. 2010). This study indicated that a conventional step-up strategy and the presence of mucosal damage predict a relapse in years 3 and 4. In an observational Danish single-center retrospective study, 53 patients with CD and 28 patients with UC stopped IFX while in steroid-free remission, and these patients were monitored for up to 10 years. In this cohort, 61% of patients with CD remained in remission at one year, the proportion dropping to 50% and 12%, at 2 and 10 years, respectively. Of the patients with UC, 75% were still in remission at one year and 40% after 4.5 years (Steenholdt et al. 2012). CD patients with longer disease duration at the onset of TNFα-blocking therapy relapsed more often, but as in UC, no risk factors for relapse could be identified. Rismo and
coworkers (2013) presented a study of 37 patients treated with either IFX or ADA with complete MH documented by colonoscopy. The median time to clinical relapse was 26 weeks, with 7 of 27 evaluated patients (26%) still in remission by week 52. Univariate analyses showed differences in mucosal cytokine levels (IL-17A and TNFα expression) between patients relapsing and those sustaining remission. In an observational prospective cohort, IFX or ADA was discontinued in 121 patients in clinical remission after one year of therapy. In this study, only one-third of patients agreed to undergo colonoscopy, which showed MH in only 35%. During a one year follow-up, 45% of patients had clinical relapse and restarted biologics (Molnar et al. 2013). The authors found a dose intensification during the one year course of anti-TNF therapy, previous biological therapy, smoking and steroid therapy at the time of induction, elevated CRP level at the beginning and end of therapy, and male gender to be predictors of a need for restarting biological therapy. In a recently published study of 51 UC patients in clinical remission, 35% needed to be retreated with IFX within one year after treatment cessation (Farkas et al. 2013).

Existing guidelines have concluded that due to limited evidence no recommendations can be made regarding in whom and when to discontinue TNFα-blocking therapy after attaining clinical remission (Dignass et al. 2010, 2012a, D’Haens et al. 2011). A multidisciplinary European expert panel (European Panel on the Appropriateness of Crohn’s Disease Treatment II, EPACT-II) considered discontinuation of TNFα-blocking therapy in CD to be appropriate after 4 years, but also after 2 years of therapy if the patient was in DR. Nonetheless, in clinical practice, the decision on whether to continue or discontinue treatment in IBD patients in clinical or endoscopic remission is still mostly based on assessment of the patient’s individual risks and benefits.

7.1.2.4 Reinitiation of TNFα-blocking therapy

The existing data on re-initiation of TNFα-blocking therapy confirm the response to be effective and well tolerated. In the STORI trial, patients were retreated after a mean drug holiday of 6.6 months (Louise et al. 2012). After only two infusions of IFX, 88% of patients were in clinical remission and 98% had a clinical response. As there is a risk of immunization resulting in hypersensitivity reactions when restarting medication after a drug-free interval, it is important to note that none of the patients were positive for ATI before retreatment. Also Steenholdt and coworkers (2012) report an excellent outcome after retreatment; 96% CD patients and 71% of UC patients achieved complete clinical remission. Reassuringly, Molnár and coworkers (2013) reported that retreatment with biologics was effective in 55% of CD patients and 94% of UC patients after clinical relapse resulting from discontinuation of TNFα-blocking therapy.
### 7.2 Surgical therapy

As a general rule, surgery is the appropriate choice of treatment in UC when medical treatment options have failed, particularly in severe acute colitis or chronically active steroid-dependent colitis, or when the risk of malignancy is high, i.e. in dysplasia or cancer of the colon (Mowat et al. 2011). Despite advances in medical treatment of UC, 25-30% of patients will require surgery at some point in their life. The cumulative 10-year colectomy rate is about 10% (Solberg et al. 2009). The two main options for surgical treatment for UC are panproctocolectomy with formation of an end ileostomy and restorative proctocolectomy (RPC) with an ileal pouch-anal anastomosis (IPAA). The latter is the standard care in elective surgery for UC (Digass et al. 2012a). Proctocolectomy with ileostomy is recommended for patients with impaired sphincter function, advanced age, significant comorbidities, or distal rectal cancer (Cohen et al. 2005). In some rare cases, a total colectomy with an ileorectal anastomosis is an option.

Surgery in CD should be limited to the disease-related complications, such as strictures and fistulae, and emergencies, including perforations, intestinal obstruction, and bleeding. Surgery is the inevitable outcome for approximately 50% of patients with ileocaecal CD in the first 10 years after diagnosis, and all CD patients have a 70-80% lifetime risk of surgery (Carter et al. 2004). In localized colonic disease, limited resection of the affected segment is advised. However, in multi-segment colonic disease a subtotal colectomy with ileorectal anastomosis should be considered. Ileal pouch-anal anastomosis is not usually recommended in CD due to a high risk of complications and pouch failure. In ileal CD, stricturoplasty plays a central role. Although immunosuppressants have been used more frequently during the last 25 years, no significant decrease in surgery rates has been reported (Cosnes et al. 2005, Domenech et al. 2010). Even during the era of TNFα-blocking therapy, findings for the need for surgery have been controversial (Nguyen et al. 2011, Ehteshami-Afshar et al. 2011). A majority of patients achieve symptom relief after primary surgery, however, up to 44% of patients have needed second surgery during a 7-year follow-up (Larson and Pemberton 2004). Surgery should be considered a treatment option for CD patients and should be discussed with patients at an early stage (Dignass et al. 2010).
AIMS OF THE STUDY

The aims of Studies I-IV were as follows:

1. To determine how often deep remission is achieved in IBD patients on TNFα-blocking therapy in everyday clinical practice (Study II). This information is essential when considering the possibility of discontinuing TNFα-blocking therapy in IBD patients.

2. To assess the relapse rate and predictive factors for relapse after cessation of maintenance therapy with TNFα-blocking agents in IBD patients in deep remission (Study III).

3. To evaluate the role of fecal calprotectin in predicting disease outcome of patients on TNFα-blocking therapy (Study I).
   in monitoring IBD patients after stopping TNFα-blocking therapy once patients attain deep remission (Study IV).
PATIENTS AND METHODS

1 Patients

1.1 Retrospective studies (I and II)

1.1.1 Patients in Study I

This study included all patients treated during the period April 2005 to April 2010 at the Division of Gastroenterology, Helsinki University Central Hospital, for active luminal disease who had an elevated FC level at baseline and a documented FC concentration after induction with TNFα-blocking agents. Data were analyzed retrospectively for 60 IBD patients (34 CD, 26 UC/IBDU). For induction patients received IFX 5 mg/kg either at weeks 0, 2, and 6 or at weeks 0 and 8 (16 CD, 26 UC) or ADA 160 mg at week 0, 80 mg at week 2, and thereafter 40 mg every other week or 80 mg at week 0 and thereafter 40 mg every other week (18 CD). After the induction, anti-TNF therapy was continued in all responders as a scheduled maintenance therapy for at least one year if not relapsing earlier. Based on the post-induction FC concentration, patients were grouped as normal post-induction FC (FC < 100 μg/g) or elevated post-induction FC (FC ≥ 100 μg/g).

1.1.2 Patients in Study II

The data were collected retrospectively at eight gastroenterological units in Finland. We included 252 IBD patients (183 CD, 62 UC, and 7 IBDU) treated with a scheduled anti-TNF therapy for at least 11 months during 2010 and 2011. Altogether 126 CD patients (69%) and 63 UC/IBDU (91%) patients were on concomitant immunomodulators (azathioprine, 6-mercaptopurine, or metotrexate). An ileocolonoscopy was mandatory. Because of its small size, the subgroup IBDU with 7 patients was included in the UC group. Only 20% (37 patients) of CD patients were treated for fistulizing disease, and the remaining 79% (145 patients) for luminal CD. In addition, in two patients with pediatric CD onset, the main indication for TNFα-blocking therapy had been delayed growth. Of patients with UC or IBDU, 45% (n=35) had a steroid-refractory disease and 55% (n=38) a steroid-dependent disease.

Clinical characteristics of patients from Studies I and II are shown in Table 14.
<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n=60$</td>
<td>$n=252$</td>
</tr>
<tr>
<td></td>
<td>(CD 34, UC 26)</td>
<td>(CD 34, UC 62, IBDU 7)</td>
</tr>
<tr>
<td><strong>Sex, male/female</strong></td>
<td>DC: 19/15</td>
<td>DC: 85/98</td>
</tr>
<tr>
<td></td>
<td>UC: 9/17</td>
<td>UC: 37/25</td>
</tr>
<tr>
<td></td>
<td>IBDU: 5/2</td>
<td></td>
</tr>
<tr>
<td><strong>Age at diagnosis, years, median (range)</strong></td>
<td>CD: 21 (12–50)</td>
<td>CD: 21 (2–57)</td>
</tr>
<tr>
<td></td>
<td>UC: 26 (16–46)</td>
<td>UC: 25 (8–72)</td>
</tr>
<tr>
<td></td>
<td>IBDU: 18 (17–65)</td>
<td></td>
</tr>
<tr>
<td><strong>Age at induction, years, median (range)</strong></td>
<td>CD: 30 (19–52)</td>
<td>CD: 32 (12–69)</td>
</tr>
<tr>
<td></td>
<td>UC: 29 (18–57)</td>
<td>UC: 31 (13–75)</td>
</tr>
<tr>
<td></td>
<td>IBDU: 27 (22–68)</td>
<td></td>
</tr>
<tr>
<td><strong>Duration of disease, years, median (range)</strong></td>
<td>CD: 7 (1–27)</td>
<td>CD: 8 (&lt;1–33)</td>
</tr>
<tr>
<td></td>
<td>UC: 3 (1–24)</td>
<td>UC: 5 (&lt;1–34)</td>
</tr>
<tr>
<td></td>
<td>IBDU: 5 (&lt;1–9)</td>
<td></td>
</tr>
<tr>
<td><strong>Localization of CD, $n$ (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum (L1)</td>
<td>7 (20.5%)</td>
<td>14 (8%)</td>
</tr>
<tr>
<td>Colon (L2)</td>
<td>7 (20.5%)</td>
<td>55 (30%)</td>
</tr>
<tr>
<td>Ileocolon (L3)</td>
<td>17 (50%)</td>
<td>106 (58%)</td>
</tr>
<tr>
<td>Upper GI</td>
<td>1 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>L1 + upper GI</td>
<td>1 (3%)</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>L2 + upper GI</td>
<td>0</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>L3 + upper GI</td>
<td>1 (3%)</td>
<td>4 (2%)</td>
</tr>
<tr>
<td><strong>Behavior of CD, $n$ (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory (B1)</td>
<td>9 (26%)</td>
<td>86 (47%)</td>
</tr>
<tr>
<td>Stricturing (B2)</td>
<td>16 (47%)</td>
<td>45 (25%)</td>
</tr>
<tr>
<td>Penetrating (B3)</td>
<td>2 (6%)</td>
<td>20 (11%)</td>
</tr>
<tr>
<td>B1 + perianal disease</td>
<td>4 (12%)</td>
<td>11 (6%)</td>
</tr>
<tr>
<td>B2 + perianal disease</td>
<td>1 (3%)</td>
<td>13 (7%)</td>
</tr>
<tr>
<td>B3 + perianal disease</td>
<td>2 (6%)</td>
<td>8 (4%)</td>
</tr>
<tr>
<td><strong>Localisation of UC/IBDU, $n$ (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancolitis</td>
<td>3 (12%)</td>
<td>45 (65%)</td>
</tr>
<tr>
<td>Proctosigmoiditis</td>
<td>23 (88%)</td>
<td>24 (35%)</td>
</tr>
<tr>
<td>Proctitis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Current smoking; no/yes</strong></td>
<td>CD: 25/9</td>
<td>UC: 23/3</td>
</tr>
<tr>
<td><strong>Anti-TNF therapy used, $n$ (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infliximab</td>
<td>42 (70%)</td>
<td>177 (70%)</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>18 (30%)</td>
<td>75 (30%)</td>
</tr>
</tbody>
</table>

CD = Crohn’s disease; UC = ulcerative colitis; IBDU = inflammatory bowel disease unclassified
GI = gastrointestinal
1.2 Prospective studies (III and IV)

1.2.1 Patients in Study III

Between February 2010 and June 2013, 52 adults with IBD (17 CD, 35 UC/IBDU) were recruited to this prospective multicenter study. All patients had been treated with TNFα-blocking therapy for at least 11 months and were in DR, i.e. clinical, endoscopic, and FC-based (<100 μg/g) remission, at inclusion. In addition, all patients had been in corticosteroid-free remission over the 6 months previous to study inclusion. Of the patients, 13 CD (76%) and 30 UC/IBDU (85%) were on concomitant immunomodulators. Patients with a history of escalation of TNFα-blocking agents during the previous 6 months, perianal disease with no other effective medication available, history of relapse after TNFα-blocking agent withdrawal, severe arthritis as a concomitant indication for TNFα-blocking therapy, and pregnancy were excluded. Follow-ups with assessment of clinical activity were performed every 4 weeks up to 6 months and thereafter every second month until relapse occurred of the study. In case of relapse, monitoring continued up to 12 months after the restart of treatment. Endoscopic assessment of disease activity was performed at 4 and 12 months after drug withdrawal. Patients were asked to provide a stool sample for FC and blood samples for hemoglobin, white blood cell count, platelet count, and CRP levels at baseline and at 2, 4, 8, and 12 months after withdrawal. During the prospective follow-up the maintenance therapy was unaltered.

1.2.2 Patients in Study IV

Of the 52 patients in Study III, 49 (16 CD, 33 UC/IBDU) participated in Study IV. Three patients (one CD, two UC) were excluded due to lack of follow-up FC samples.

Clinical characteristics of patients from Studies III and IV are shown in Table 15.
<table>
<thead>
<tr>
<th></th>
<th>Study III (n=52)</th>
<th>Study IV (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(CD 17, UC/IBDU 35)</td>
<td>(CD 16, UC/IBDU 33)</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>CD: 8/9</td>
<td>CD: 85/98</td>
</tr>
<tr>
<td></td>
<td>UC/IBDU: 20/15</td>
<td>UC/IBDU: 37/25</td>
</tr>
<tr>
<td>Age at diagnosis, years, median (range)</td>
<td>CD: 22 (13–42)</td>
<td>CD: 23 (13–42)</td>
</tr>
<tr>
<td></td>
<td>UC/IBDU: 26 (8–45)</td>
<td>UC/IBDU: 26 (8–45)</td>
</tr>
<tr>
<td>Age at induction, years, median (range)</td>
<td>CD: 32 (15–52)</td>
<td>CD: 32 (15–52)</td>
</tr>
<tr>
<td></td>
<td>UC/IBDU: 32 (13–58)</td>
<td>UC/IBDU: 32 (13–58)</td>
</tr>
<tr>
<td>Duration of disease, years, median (range)</td>
<td>CD: 10 (3–26)</td>
<td>CD: 9 (3–25)</td>
</tr>
<tr>
<td></td>
<td>UC/IBDU: 6 (1–35)</td>
<td>UC/IBDU: 6 (1–35)</td>
</tr>
<tr>
<td>Localization of CD, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum (L1)</td>
<td>1 (6%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Colon (L2)</td>
<td>4 (24%)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>Ileocolon (L3)</td>
<td>12 (70%)</td>
<td>11 (69%)</td>
</tr>
<tr>
<td>Behavior of CD, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory (B1)</td>
<td>11 (65%)</td>
<td>10 (63%)</td>
</tr>
<tr>
<td>Stricturing (B2)</td>
<td>4 (25%)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>Penetrating (B3)</td>
<td>1 (6%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>B1 + perianal disease</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B2 + perianal disease</td>
<td>1 (6%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>B3 + perianal disease</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Localization of UC/IBDU, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancolitis</td>
<td>20 (57%)</td>
<td>19 (65%)</td>
</tr>
<tr>
<td>Proctosigmoiditis</td>
<td>15 (43%)</td>
<td>14 (35%)</td>
</tr>
<tr>
<td>Proctitis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Current smoking: no/yes</td>
<td>CD: 25/9</td>
<td>CD: 25/9</td>
</tr>
<tr>
<td></td>
<td>UC: 23/3</td>
<td>UC: 23/3</td>
</tr>
<tr>
<td>Duration of TNFalpha-blocking therapy before cessation of therapy, months, median, (range)</td>
<td>CD: 32 (11–72)</td>
<td>CD: 34 (11–70)</td>
</tr>
<tr>
<td></td>
<td>UC/IBDU: 14 (11–78)</td>
<td>UC/IBDU: 12 (11–77)</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>CD: 26 (16–36)</td>
<td>CD: 26 (16–36)</td>
</tr>
<tr>
<td>History of anti-TNF therapy, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infliximab</td>
<td>CD: 12 (71)</td>
<td>CD: 11 (69)</td>
</tr>
<tr>
<td></td>
<td>UC/IBDU: 35 (100)</td>
<td>UC/IBDU: 33 (100)</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>CD: 5 (29)</td>
<td>CD: 5 (31)</td>
</tr>
</tbody>
</table>

CD = Crohn’s disease; UC = ulcerative colitis; IBDU = inflammatory bowel disease unclassified
2 Methods

2.1 Clinical scoring

In retrospective Study I, clinical activity indices were mostly collected from research files, but in some cases they were scored according to data available in patient records. Clinical disease activity was assessed with HBI (Harvey and Bradshaw 1980) in CD and with partial Mayo score (total Mayo score without endoscopy) (D’Haens et al. 2007) in UC at baseline, after induction with anti-TNF antagonists, and at one year. In Study II, the physicians’ global assessment (PGA) score was used for assessment of disease activity at the time of ileocolonoscopy. Patients with GI symptoms indicating active IBD scored 1 and nonsymptomatic patients 0. In prospective studies III and IV, HBI served as a measure for clinical disease activity in CD and partial Mayo score in UC/IBDU. In Studies I, III, and IV, clinical remission was defined as HBI < 4, mildly active disease as HBI 4-7, a moderately active disease as HBI 8-16, and severely active disease as HBI ≥ 17 (Best et al. 2006). Clinical relapse was defined as HBI ≥ 8 or an increase of > 3 points in HBI to at least 5 points. In partial Mayo score, 0 was defined as remission, 1-3 as mildly active disease, 4-6 as moderately active disease, and > 7 as severely active disease. With 0 was defined as remission, clinical relapse was defined as a partial Mayo score ≥ 3 (Schroeder et al. 1987).

2.2 Endoscopic scoring

In CD, endoscopic findings were graded according to the Simple Endoscopic Score for Crohn’s disease (SES-CD) (Dapero et al. 2004), and in UC according to the Mayo endoscopic score (D’Haens et al. 2007). SES-CD 0-2 was defined as remission, 3-6 as mildly active disease, 7-15 as moderately active disease, and ≥16 as severely active disease (Moskovitz et al. 2007). For the Mayo endoscopic subscore, endoscopic findings were graded as normal (0), mild (1), moderate (2), or severe (3); a subscore of 0-1, was defined as remission and a subscore of >2 as active disease (D’Haens et al. 2007). Ileocolonoscopies were preformed and endoscopic findings scored in participating units as part of routine clinical practice.

2.3 Histological scoring (II and IV)

Ileocolonoscopies were performed in all four studies and random biopsy specimens were taken from five different segments: the ileum, the right, transverse, and left colon, and the rectum. During all endoscopies biopsies were collected from the most severely diseased areas or, if no lesions were found, from random sites of each segment. In Study II, we graded histological findings according to the most severely diseased areas, 0 representing normal areas and 1 active inflammation. In Study IV, a single experienced GI pathologist scored the histological findings according to the scoring system developed for histological abnormalities in CD (D’Haens et al. 1998) and UC (D’Haens et al. 2007). The scoring system is described in detail in Study IV.
2.4 Fecal calprotectin and blood tests

Fecal calprotectin was measured by a quantitative enzyme immunoassay (the CALPRO® calprotectin ELISA test (ALP; Calpro AB, Lysaker, Norway)) and FC concentration < 100 µg/g was quoted as normal (von Roon et al 2007). Blood tests, such as blood count, ESR, and CRP, were performed as part of routine clinical follow-up and determined by normal laboratory values.

2.5 Quality of life (III)

Questionnaires on the quality of life were used in Study III. All patients were asked to fill in the Inflammatory Bowel Disease Questionnaire (IBDQ) and 15D at baseline, at the time of possible relapse, and at the time of the ileocolonoscopies. The IBDQ was used under license from McMaster University, Hamilton, Ontario, Canada. The IBDQ and 15D are described in detail in Study III.

2.6 Statistics

For data analyses in all studies, we used Statistical Package for the Social Sciences (SPSS version 17.0 and PASW 18) for Windows software (SPSS, Chicago, IL, USA). The results were given as percentages, as median and range, or as mean and standard deviation (SD). In Study I, a power calculation was performed. The Wilcoxon signed-rank test was used to test the differences between related variables (Study I), and the Mann-Whitney U-test (Studies I-IV) and T-test (Studies III and IV) to assess the differences between independent variables. Fisher’s exact test (Studies I and III) was used to determine differences in binary variables, and the Chi square test (Study II) to determine differences between categorical variables. Normality of the continuous variables was evaluated with the Kolmogorov-Smirnov test (Study III). The Cox regression of proportional hazards was used to calculate univariate hazard ratios for categorical and continuous variables (Study III). Kaplan-Meier survival analysis served to estimate relapse-free survival rates, and the log rank test to determine differences between the groups (Study III and IV). Receiver-operator characteristic (ROC) curve analysis was used to calculate the cut off value for FC to predict the outcomes (Study I and IV). The optimized cutoff level offered the best combination of sensitivity and specificity. In Study IV, we used the general estimating equation model to calculate the estimated FC level at relapse in the various groups. FC values were calculated at individual time-points and presented as medians and range. Significance was set at $p < 0.05$. 
2.7 Ethical considerations

The study protocols of Studies III and IV and all documents were approved by the Ethics Committee of Helsinki University Central Hospital (number 348/13/03/01/2009) and the ethics committees of each participating university central hospital. No ethics committee approval was sought for Studies I and II because these studies comprised merely a retrospective reviewing of patients' medical records. Research study permission number 195/2009 was received from the Department of Medicine, Helsinki University Central Hospital in December 2009. For Studies III and IV, all patients gave their written consent prior to participation.
RESULTS

1 Achievement of deep remission in IBD with TNFα-blocking therapy

Study II
Of 252 patients, 168 (67%) were in clinical remission and 122 (48%) in DR after a median of 23 months of TNFα-blocking therapy (range 11-147). Up to 63% (116/183) of CD patients and 75% (52/69) of UC patients achieved clinical remission, whereas 43% (79/183) of CD patients and 62% (43/69) of UC patients achieved DR (Figure 1). No significant difference was found in achieving clinical remission between CD and UC patients (p=0.072), but UC patients achieved DR significantly more often than CD patients (p=0.007). Of all CD patients in DR, 75% were also in histological remission, whereas in UC the rate was 93% (p=0.001). Concomitant histological remission with DR occurred in 59 CD patients (32%) and 40 UC patients (58%). The significance of combination therapy with azathioprine, 6-mercaptopurine, and methotrexate on the DR rates was also evaluated; however, no significant differences were found in inducing DR in patients on combination therapy compared with patients on monotherapy (all patients p = 0.214, CD p = 0.845, UC p = 0.107). CD patients receiving IFX achieved DR slightly more often than patients on ADA therapy (46% vs. 39%), but because of the relatively heterogeneous study population, no direct comparison between the two regimens was conducted.

Based on clinical judgement, in 43% (53/122) of patients in deep remission TNFα-blocking therapy was withdrawn. Discontinuation of TNFα-blocking therapies was tried for the first time in all of these patients. In the remaining 69 patients (57%) TNFα-blocking therapy was continued because of a disease with no other effective medication available (n = 19), fistulating disease (n = 6), concomitant rheumatoid disease (n = 4), high FC level (n = 6), history of severe disease (n = 19), or previous unsuccessful withdrawal of TNFα-blocking therapy.
Figure 1. Achievement of clinical and deep remission in IBD with TNFα-blocking therapy.

2 Discontinuation of TNFα-blocking therapy in IBD

Study III
A total of 52 patients (17 CD, 30 UC, 5 IBDU) were recruited in Study III. Because of its small size, the subgroup IBDU with five patients was combined with the UC group. During the follow-up phase one UC patient dropped out at 6 months while in clinical remission. Of the remaining 51 patients, 5/17 (29%) CD and 12/34 (35%) UC/IBDU patients relapsed after a median follow-up of 13 (range 12-15) months and 34 of patients (67%) remained in clinical remission (Figure 2). Twenty-nine (85%) of these patients were also in endoscopic remission. In relapers, ten patients (19%) (3 CD, 7 UC) experienced both clinical and endoscopic relapse, two CD patients (4%) a nonsymptomatic severe endoscopic relapse, and five UC patients (10%) a clinical relapse with mild endoscopic activity (Mayo score 1) not fulfilling the predefined criteria for endoscopic relapse (endoscopic Mayo score ≥ 2), but were considered relapers because of severe clinical symptoms. Relapses occurred at a median of 6 (range 2.5-15) months after cessation of TNFα-blocking therapy. Interestingly, no significant difference was found between CD and UC/IBDU in the relapse rate (p = 0.896).
In this study, we also evaluated the odds of a reduction in quality of life scores in relapsers. As expected, both 15D score (0.955 [0.879 – 1.0] versus 0.853 [0.745 - 0.938], p < 0.001) and IBDQ score (193 [164 - 223] versus 142 [102 - 183], p < 0.001) were significantly lower at relapse than at baseline. Both scores were equal at baseline and at 12 months after the restart of TNFα-blocking therapy (p = 0.705 and p = 1.0, respectively).
Risk factors for relapse
The risk factors were calculated by the Cox model. No specific factor, such as gender, age at diagnosis, disease duration, localization or behavior of the disease, previous surgery, smoking, chosen TNFα-blocking therapy, duration of TNFα-blocking therapy, concomitant medications, quality of life score, or haemoglobin or CRP levels at the time of discontinuation, was found to predict the relapse (Table 16).

Table 15. Risk factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relapse (n=17)</th>
<th>Remission (n=34)</th>
<th>HR</th>
<th>95% CI</th>
<th>P – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex (%)</td>
<td>9 (53)</td>
<td>19 (56%)</td>
<td>0.89</td>
<td>0.34/2.31</td>
<td>0.808</td>
</tr>
<tr>
<td>Positive family history</td>
<td>2 (12%)</td>
<td>8 (24%)</td>
<td>0.44</td>
<td>0.10/1.92</td>
<td>0.275</td>
</tr>
<tr>
<td>Age at diagnosis, median years (range)</td>
<td>22 (8-44)</td>
<td>26 (16-45)</td>
<td>0.98</td>
<td>0.92/1.03</td>
<td>0.387</td>
</tr>
<tr>
<td>Age at induction of TNFα-blocking therapy, median years</td>
<td>32 (13-52)</td>
<td>32 (17-58)</td>
<td>0.98</td>
<td>0.94/1.03</td>
<td>0.510</td>
</tr>
<tr>
<td>Duration of disease, median years (range)</td>
<td>8 (2-26)</td>
<td>7 (1-35)</td>
<td>1.01</td>
<td>0.95/1.07</td>
<td>0.816</td>
</tr>
<tr>
<td>Duration of TNFα-blocking therapy, mean months (range)</td>
<td>26 (10.71)</td>
<td>22 (10.77)</td>
<td>1.01</td>
<td>0.98/1.03</td>
<td>0.572</td>
</tr>
<tr>
<td>Localization of UC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proctosigmoiditis</td>
<td>5 (45%)</td>
<td>10 (45%)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extensive colitis</td>
<td>6 (55%)</td>
<td>12 (55%)</td>
<td>0.99</td>
<td>0.30/3.23</td>
<td>0.981</td>
</tr>
<tr>
<td>Localization of CD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>1 (17%)</td>
<td></td>
<td>1.00</td>
<td></td>
<td>0.365</td>
</tr>
<tr>
<td>Ileocolon</td>
<td>4 (67%)</td>
<td>9 (75%)</td>
<td>0.20</td>
<td>0.02/1.98</td>
<td>0.167</td>
</tr>
<tr>
<td>Colon</td>
<td>1 (17%)</td>
<td>3 (25%)</td>
<td>0.18</td>
<td>0.01/3.23</td>
<td>0.247</td>
</tr>
<tr>
<td>Disease behavior (Mb Crohn)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory (B1)</td>
<td>5 (83%)</td>
<td>7 (58%)</td>
<td>1.00</td>
<td></td>
<td>0.970</td>
</tr>
<tr>
<td>Strictureing (B2)</td>
<td>1 (17%)</td>
<td>3 (25%)</td>
<td>0.58</td>
<td>0.07/4.99</td>
<td>0.622</td>
</tr>
<tr>
<td>Penetrating (B3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2 + perianal</td>
<td>1 (8%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous surgery</td>
<td>1 (6%)</td>
<td>7 (21%)</td>
<td>0.30</td>
<td>0.04/2.25</td>
<td>0.240</td>
</tr>
<tr>
<td>Smoker or previous smoker</td>
<td>4 (24%)</td>
<td>16 (47%)</td>
<td>0.43</td>
<td>0.14/1.33</td>
<td>0.144</td>
</tr>
<tr>
<td>TNFα-blocking therapy used</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infliximab</td>
<td>16 (94%)</td>
<td>30 (88%)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adalimumab</td>
<td>1 (6%)</td>
<td>4 (12%)</td>
<td>0.56</td>
<td>0.07/4.23</td>
<td>0.574</td>
</tr>
<tr>
<td>Concomitant</td>
<td>14 (82%)</td>
<td>29 (85%)</td>
<td>0.91</td>
<td>0.26/3.16</td>
<td>0.877</td>
</tr>
<tr>
<td>immunosuppressive therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP at discontinuation, mean (range)</td>
<td>2.6 (1-3)</td>
<td>3.8 (1-16)</td>
<td>0.81</td>
<td>0.57/1.15</td>
<td>0.231</td>
</tr>
<tr>
<td>Hemoglobin at discontinuation, mean (range)</td>
<td>142 (127-171)</td>
<td>138 (109-167)</td>
<td>1.02</td>
<td>0.98/1.05</td>
<td>0.350</td>
</tr>
<tr>
<td>IBDQ at discontinuation, mean (range)</td>
<td>193 (164-223)</td>
<td>197 (151-219)</td>
<td>0.99</td>
<td>0.96/1.02</td>
<td>0.425</td>
</tr>
<tr>
<td>ESD at discontinuation, mean (range)</td>
<td>0.96 (0.879-1)</td>
<td>0.95 (0.844-1)</td>
<td>3.72</td>
<td>0.00/4.90</td>
<td>0.827</td>
</tr>
</tbody>
</table>

HR = hazard ratio; CD = Crohn’s disease; UC = ulcerative colitis; CRP = C-reactive protein; IBDQ = inflammatory bowel disease questionnaire
Restart of TNFα-blocking therapy

TNFα-blocking therapy was restarted in 15 patients and they were followed up for approximately 12 (range 9-14) months. No infusion reactions or serious adverse events were reported during the follow-up. One UC patient without response to restart of infliximab underwent colectomy less than 2 months after the restart of infliximab. At 3 months, 14 patients (93%) were in clinical remission, and at 12 months all four CD patients and nine of ten UC patients (90%) were in clinical remission. Endoscopic assessment of disease activity was performed on three CD patients and nine UC patients at 12 months. Ileocolonoscopies showed mild activity in all three CD patients, but endoscopic remission in all UC patients. No tendency towards an increase in clinical or endoscopic activity indices or a decrease in IBDQ or 15D scores before the relapse was found.

3 Long-term treatment response and outcome after cessation of TNFα-blocking therapy

3.1 Fecal calprotectin as a predictor of long-term treatment response

Study I

Sixty patients with elevated baseline FC (median 810 µg/g, range 103–12,258 µg/g) were recruited. After induction with TNFα-antagonists, median FC level decreased to 97 µg/g (range 0–5859, p < 0.001). Patients were divided into two groups based on FC levels after induction therapy (Figure 3). Of 60 patients, 31 (52%) had normal FC after induction therapy, whereas 29 (48%) had an elevated FC. In this latter group, patients in clinical remission had lower median FC concentrations after induction therapy than those with clinically active disease (204 µg/g, range 116–670 vs. 496 µg/g, range 123–2896, p = 0.025). In the normal FC group, after induction therapy, 27/31 (84%) showed FC decline more than 75% and up to 82% of these patients were in clinical remission at one year, whereas only 38% of patients with an elevated post-induction FC had achieved clinical remission (p = 0.002). At approximately one year, 48 patients were on maintenance therapy. Of these 48 patients, 37 (27 CD, 10 UC) were in clinical remission and 8 (7 CD, 1 UC) had clinically active disease, CD patients significantly more often than UC patients (p = 0.001). Three UC patients underwent colectomy. Of 31 patients in the normal post-induction calprotectin group, 26 (84%) were in clinical remission, whereas only 11 patients (38%) in the elevated post-induction FC group were in clinical remission (p<0.0001) (Figure 4). Of 48 patients, 25 had provided a stool sample for FC at one year, 17 in the normal post-induction FC group and 8 elevated in the post-induction FC group. In the normal post-induction FC group, 13 patients (76%) showed a normal FC value, whereas only 4 patients (50%) had a normal FC in the elevated post-induction FC group. Endoscopic reassessment was performed for 38 patients (27 CD, 11 UC) at one year. Endoscopic remission (SES-CD < 3 or Mayo endoscopic subscore ≤ 1) was discovered more often in patients with a normal post-induction FC; the difference was not significant (17/23, 74% versus 7/15, 47%). Moreover, in the normal post-induction FC group, 17
patients (62%) were in both clinical and endoscopic remission, while only seven patients (33%) achieved clinical and endoscopic remission in the elevated post-induction FC group.

Figure 3. Flow chart of Study I (data adapted from Molander et al. 2012).
Figure 4. Kaplan-Meier survival curve showing the risk of relapse in relation to FC levels below and above 100 µg/g. P value was calculated by the log-rank test ($p<0.001$).

The ROC curve in Study I was constructed to predict the long-term outcome according to the post-induction FC level. The FC value of 139 µg/g, with a sensitivity of 72%, a specificity of 80%, and AUC 0.838 [0.724-0.952], was optimized as the best cut-off for predicting relapse at one year. Chosen anti-TNF antagonists (OR 0.35; 95% CI 0.10–1.23, $p = 0.147$), smoking (OR 1.19, 95% CI 4.32–0.33, $p = 1$), or gender (OR 0.33; 95% CI 0.11–1.00, $p = 0.064$) did not have a significant impact on the clinical remission rate. An additional ROC curve was constructed to predict the positive outcome according to decline of the FC from baseline to post-induction assessment. A decline of 88% in FC gave the maximum sensitivity (87%), specificity (65%), and AUC (0.771, 0.652–0.890) for predicting clinical remission at one year.
3.2 Fecal calprotectin as a predictor of relapse

Study II
In Study II, FC measurements were available for 163 of 252 patients. Median FC level was significantly lower for the 79 patients in DR than for the 84 patients with active disease (50 µg/g, range 1–722 vs. 288 µg/g, range 6–4190, p < 0.0001). Of all patients, FC was normal (<100 µg/g) in 88. In the DR, 63 patients (72%) had normal FC levels, but in the active disease group, FC was normal in only 16 patients (21%) (p < 0.0001).

Study IV
Study IV included 49 patients in deep remission with TNFα-blocking therapy. After discontinuing of anti-TNF agents, 34 patients (69%, 12 CD, 22 UC) remained in remission during the one-year follow-up and 15 (31%, 4 CD, 11 UC) experienced either clinical or endoscopic relapse (9 patients; 2 CD, 7 UC), only endoscopic relapse (2 CD), or clinical relapse with mild endoscopic activity (4 UC). No clinical relapses without signs of endoscopic activity were found.

FC concentrations and relapse risk (Study IV)
Stool samples for FC measurements were collected to evaluate whether elevated FC after discontinuing TNFα-blocking therapy can predict clinical or endoscopic relapse. A significantly higher median baseline FC concentration was seen in patients with relapse already 6 (120 µg/g, 0–431, n = 6, p = 0.0029), 4 (108 µg/g, 7–650, n = 8, p = 0.0056), and 2 months (120 µg/g, 0–1867, n = 15, p = 0.0014) before the clinical relapse. Differing from the patients with stable remission, once FC was elevated in the group of patients with relapse, it remained elevated until clinical or endoscopic relapse occurred. In patients with stable remission, the elevation in FC concentration appeared to be transient in the majority (18/22, 82%). With ROC analysis, we evaluated the best cut-off value for FC to predict relapse. The cut-off of >140 µg/g could predict relapse with 79% specificity and 53% sensitivity 6 months before the relapse. Furthermore, ROC analyses indicated that FC > 200 µg/g measured 2-4 months before clinical relapse and FC > 199 µg/g measured 4-6 months before relapse could predict relapse with 84% and 94% specificity and 50% and 53% sensitivity, respectively. No significant difference was found in relapse rate with patients who had FC 50 µg/g or FC 50–100 µg/g at baseline (p = 0.325). The general estimating equation model showed that at relapse patients with relapse had higher FC levels (estimated 199 µg/g, 95% CI 103–386 µg/g) than nonrelapsers at the end of the follow-up (49 µg/g, 95% CI 35–69 µg/g).

Fecal calprotectin and histological findings (Study IV)
In Study IV, we also evaluated the correlation of FC and histological findings, as well as the impact of histological inflammation on relapse risk. Endoscopic assessment was performed in all 49 patients at baseline, in 41 patients at 4 months, and in 31/34 patients still in remission at 12 months. At baseline, 40 patients (82%) showed no signs of histological inflammation in specimens, whereas mild infiltration of neutrophils in the lamina propria or in the
epithelium appeared in four patients (8%) and moderate inflammation in five patients (10%). No significant difference was found in the relapse rate in patients with acute or chronic inflammatory infiltrate at baseline compared with those who had no signs of inflammation in biopsy specimens ($p = 0.221$). Since there is no validated histological score for remission or relapse, we defined histological remission as no neutrophils in the lamina propria or in the epithelium. Histological relapse was defined as the sum of these two scores ≥ 1. The median histology score in patients who relapsed was equal to that in patients who remained in remission ($p = 0.324$). Twelve months after cessation of TNFα-blocking therapy, the majority (16/18, 89%) of patients with normal histological findings in specimens had normal ($<100 \mu g/g$) FC concentration (34 µg/g, range 0–178), whereas only 40% of patients with mild inflammatory activity had normal FC levels (156 µg/g, range 57–340, n = 6). Comparing the levels of FC in patients with or without inflammatory activity, FC was significantly higher in active disease ($p = 0.016$).
DISCUSSION

1 Concept of deep remission and TNFα-blocking therapy in IBD

In the era of TNFα-blocking therapy, MH has become a generally accepted and desirable endpoint in the treatment of IBD. In addition, it seems to be an important predictor of treatment outcome. However, it is still unresolved whether complete MH i.e. deep remission, offers better treatment outcome than partial MH. Nevertheless, the ability of TNFα-blocking therapy to induce MH has been in great interest over the past decade. The results of our study demonstrate that during scheduled TNFα-blocking therapy in everyday clinical practice about half of IBD patients may achieve DR, and nearly 40% of patients achieve concomitant clinical, endoscopic, FC-based, and histological remission.

The post-hoc data of the EXTEND trial on DR in CD demonstrated that DR, defined as a combination of clinical remission (CDAI <150) and complete MH, was achievable in some 19% of patients by week 52 (Rutgeerts et al. 2012). Early MH was not a predictor of long-term MH in this study. However, sub-analysis of the EXTENT trial showed that DR at week 12 was associated with fewer hospitalizations, better quality of life, and lower healthcare costs during the first year of therapy (Colombel et al. 2011). Differing from the EXTEND trial, two Finnish studies have demonstrated that endoscopic findings at three months after the start of TNFα-blocking therapy are highly predictive of favorable outcome at one year in patients with luminal CD (af Björkesten et al. 2011, 2013). In both of these studies, endoscopic remission achieved at three months was associated with a significantly higher likelihood of endoscopic remission with scheduled maintenance therapy at one year; up to 70% and 90% of endoscopic anti-TNF induction responders were in endoscopic remission at one year. Some differences between the EXTEND trial and real life experiences may be explained by the strict inclusion criteria of the study and also by the exclusion from primary analysis of patients switched to the open label treatment group. The CHARM study, with no endoscopic data available, demonstrated a steroid-free clinical remission rate at week 56 for 29% of patients and also fewer hospitalizations and surgery (Colombel et al. 2009). In addition, the CHARM trial indicated that patients with a shorter disease duration presented better response rates to TNFα-blocking therapy. Similar results were shown also in the EXTEND trial. Response to treatment in early CD has been demonstrated to be more effective and associated with a better long-term outcome than later treatment (Foslier et al. 2007, D’Haens et al. 2008, Colombel et al. 2010, Ha et al. 2010, Baert et al. 2010). Patients with a disease duration of less than two years have shown the most favorable outcome (Sandborn et al. 2012a).

Several clinical trials and population-based studies have revealed that also patients on IFX therapy who achieve MH have better outcomes than those
who do not achieve MH (Rutgeerts et al. 2006, Lichtenstein et al. 2002 and 2004, Baert et al. 2010, D’Haens et al. 2002, Schnitzler et al. 2009). The ACCENT 1 endoscopic substudy demonstrated complete MH in nearly half of the patients by week 52, leading to significant reductions in the number of hospitalizations, surgeries, and days in intensive care units, compared with patients with only transient healing or no healing at all (Rutgeerts et al. 2006). Lichtenstein and coworkers (2002 and 2004) showed that patients achieving MH on maintenance therapy with IFX experienced fewer disease-associated complications, such as hospitalizations and surgery, and had a better quality of life. Similar results were also presented by Schnitzler and his colleagues (2009). Complete MH has also been demonstrated to lead to significantly higher steroid-free remission rates in patients with early-stage CD, for as long as four years after start of therapy (Baert et al. 2010). The efficacy of TNFα-blocking therapy in UC is mainly based on the ACT 1 trial. Moreover, Sandborn and coworkers (2009) demonstrated that IFX-treated patients achieving MH already at week 8 were less likely to have colectomy at week 54. Scheduled maintenance therapy with IFX 5 mg/kg resulted in MH in about 42% of patients by week 54 and with IFX 10 mg/kg in 47% (Rutgeerts et al. 2006, 2009). However, the decision on whether or not to continue the therapy, was based on clinical response (Hanauer et al. 2002). Regardless of some limitations in our study, the results of Study II reflect well the situation in clinical practice. In CD, nearly half of the patients in the IFX group and some 40% in the ADA group achieved DR. In UC, 63% of patients on IFX maintenance therapy achieved DR. The clinical remission rates were even higher. IFX therapy resulted in clinical remission in 66% of CD patients and 77% of UC patients, whereas ADA therapy induced clinical remission in 60% of CD patients. Based on existing data, the achievement of DR might be the only way to alter the course of IBD. However, the concept of DR requires better validation.

The impact of histological healing among IBD patients treated with TNFα-blocking therapy is to some extent unknown. Histological changes tend to lag behind clinical and endoscopic improvement, therefore having very little clinical relevance in decision-making. Over a decade ago, Sandborn and coworkers (2002) did not recommend the assessment of histological disease activity as a treatment endpoint due to uncertainty in the significance of histological disease activity and the localization of CD inflammation as well as the potential for sampling error. By contrast, more recently, D’Haens and coworkers (2007) stated that histological remission should be considered a secondary endpoint, at least in clinical trials. Additionally, they recommended the use of the existing scoring system for histological abnormalities, if measurement of histological disease activity was desired. However, the well-defined and validated endpoints for histological remission should be developed. We were able to demonstrate that a lower FC concentration in patients with clinical and endoscopic remission was strongly associated with histological remission. Nevertheless, histological inflammation at the time of cessation of TNFα-blocking therapy did not predict relapse. Therefore, unfortunately, the assessment of histological activity seems to offer no extra value in evaluating the risk of relapse.
2 Discontinuation and re-initiation of TNFα-blocking therapy in IBD

In recent years, several studies have been published on the duration of remission after discontinuation of TNFα-blocking therapy, indicating that in some CD patients withdrawal of TNFα-blocking therapy is an reasonable option (Baert et al. 2010, Waught et al. 2010, Louise et al. 2012, Steenholdt et al. 2012, Rismo et al. 2013, Molnar et al. 2013, Farkas et al. 2013). However, the existing data suggest that if patients are followed long enough, eventually all patients will experience either clinical (Regueiro et al. 2010) or endoscopic relapse (Regueiro et al. 2010, Sorrentino et al. 2010, Steenholdt et al. 2012, Rismo et al. 2013). However, none of these studies has assessed endoscopic activity during the follow-up. In addition, in the widely cited STORI trial of 115 CD patients, endoscopic remission was not an inclusion criterion, and therefore 23% of baseline CDEIS values were ≥ 3 and 34% of patients had remaining ulcers, indicating ongoing inflammatory activity at the time of cessation of therapy. Furthermore, in some patients, baseline FC measurement was elevated, indicating disease activity. Many of the relapse risk factors shown in previous studies are associated with incomplete remission with ongoing inflammatory disease activity, which may explain the lower remission rate in earlier studies than in ours, which included only patients in deep IBD remission (Baert et al. 2010, Waught et al. 2010, Louise et al. 2012, Steenholdt et al. 2012, Rismo et al. 2013, Molnar et al. 2013, Farkas et al. 2013). Moreover, due to our stringent inclusion and exclusion criteria, all patients with moderate or severe perianal CD or with severe arthritis were excluded. In theory, these stringent criteria could explain the higher remission rate. However, this is unlikely, because also in the STORI trial, patients with predominantly fistulizing perianal disease without significant luminal disease, with active fistulizing disease, or with severe extraintestinal manifestations were excluded. Until now, only one study has been published on a longer follow-up after discontinuation of therapy. Waught and coworkers (2010) reported that 35% of CD patients remained in sustained clinical remission for nearly 7 years after stopping anti-TNF agents. The maintenance of remission in IBD patients after cessation of TNFα-blocking therapy might be explained by its typical relapsing-remitting course, with outcome dependent on the individual disease course and timing of therapy interruption resulting in long-term remission. Possibly, a patient will remain in remission because interruption of therapy may coincide with the naturally occurring phase of quiescence. Thus, considering the immunological nature of IBD diseases, the development of mucosal immune dysregulation, i.e. weak immune response, by may also lead to long-term drug-free remission.

To tailor the TNFα-blocking therapy, dose increase or shortening of the therapeutic interval is an accepted strategy for patients who have lost response to anti-TNF agents (Gisbert et al. 2009b), but no studies have been designed to evaluate whether the minimal effective dose of anti-TNF agents in all IBD patients is the one recommended by the manufacturer or whether
a lower dose could be equally effective. On the other hand, low drug dosages may be associated with low drug trough levels, may cause antibody formation, and furthermore may affect drug efficacy and cause adverse reactions. However, nowadays, it is recommended to treat CD with anti-TNF agents at an earlier phase of the disease course, when patients are more responsive because of low disease burden and lower dosage of anti-TNF might be effective as explored in rheumatoid arthritis and other rheumatologic diseases.

To date, no widely accepted recommendations for discontinuing TNFα-blocking therapy have been made. Based on earlier studies, many authors do not advocate stopping TNFα-blocking therapy in patients who respond to therapy with no side-effects (Clarke et al. 2012, Peyrin-Biroulet et al. 2012). However, for economic and safety reasons, clear recommendations for discontinuing TNFα-blocking therapy are needed. A multidisciplinary European expert panel (D’Haens et al. 2010) considered discontinuing TNFα-blocking therapy to be appropriate after 4 years, but also after 2 years if the patient were in DR. The results of our study, with up to 67% of IBD patients remaining in clinical remission during the 12-month follow-up, suggest that withdrawal of TNFα-blocking therapy could already be possible after one year of therapy when patients, especially UC patients, are in DR.

The risk of immunization resulting in hypersensitivity reactions and loss of effect should be considered when restarting medication after a drug-freeintermission. According to available data (Louise et al. 2012, Steenholdt et al. 2012, Molnar et al. 2013), retreatment with TNFα-blocking therapy is well-tolerated and effective. No hypersensitivity reactions have been reported. Also in our study, all but one patient with relapse achieved clinical remission or response and three-quarters of patients achieved endoscopic remission during a median follow-up of 12 months.

3 Fecal calprotectin in monitoring TNFα-blocking therapy

A need for a reliable surrogate marker in monitoring treatment outcomes in IBD has arisen, with endoscopic assessment being time-consuming, costly, and unpleasant for the patient. FC has been shown to be a reliable surrogate marker for MH in IBD (Tibble et al. 2000b, Røseth et al. 2004). Røseth and coworkers (2004) were the first to demonstrate that MH can be determined by assessment of FC in a simple stool sample. In IBD patients in clinical and FC–based remission (FC <50 µg/g), the endoscopic findings in all but one patient were normal, and the majority of patients had no inflammatory activity in biopsies. Also a prospective study of 77 CD patients showed a significant correlation between FC and CDEIS (p < 0.001) (Sipponen et al. 2008b). The study concluded that FC is a useful surrogate marker for MH in CD patients receiving induction with TNFα-blocking agents, and FC < 200 µg/g could serve as a cut-off for endoscopically inactive disease. A single FC measurement has been shown to predict clinical relapse in IBD patients with clinically quiescent disease in several other studies (Costa et al. 2005, D’Inca
et al. 2008, Gisbert et al. 2009a, Garcia-Sanchez et al. 2010). Moreover, recent meta-analysis demonstrated a pooled sensitivity of 78% and a specificity of 73% for FC in predicting IBD relapse, especially in ileocolonic and colonic CD and UC (Mao et al. 2012). Furthermore, a large retrospective cohort of CD patients provided compelling evidence that FC measurement could be used to predict disease course because FC concentrations were significantly higher in patients with progressive CD than in those without disease progression (Kennedy et al. 2013). In this study, a normal FC concentration after the induction therapy was shown to predict better clinical outcome at one year in patients with active luminal IBD. We were the first group to demonstrate the capacity of normal FC concentration after induction with TNFα-blocking agents to predict clinical and probably endoscopic remission after 12 months of maintenance therapy with TNFα-blocking agents. These findings verify noninvasive FC test to be a useful and simple test in distinguishing endoscopically active disease from endoscopically inactive disease, and also in predicting relapse and disease outcome in quiescent IBD patients. Based on the available studies, it is not yet possible to set different cut-off values for CD and UC.

To date, no uniform consensus exists on the cut-off value of FC that predicts relapse, hence several cut-off values for predicting relapse in clinically inactive IBD patients have been reported. D’Inca and coworkers (2008) demonstrated that FC level > 130 mg/kg (µg/g) predicts clinical relapse in UC and in colonic CD, whereas the study of Garcia-Sanchez and coworkers (2010) showed a 6-fold increase in relapse rate in one year in UC with FC > 120 µg/g, and a 4-fold increase in CD with FC level > 200 µg/g at baseline. In a study published only as an abstract, normalization of FC was shown to correlate significantly with clinical response after anti-TNFα induction therapy. In that study, FC > 150 mg/kg (µg/g) after induction therapy showed an increased risk of relapse in one year. These findings are in line with our study, where an FC of 139 µg/g seems to predict relapse risk during TNFα-blocking maintenance therapy fairly well. The outcome was almost equal when comparing patients with normal FC level and those with 75% decline of FC level after induction. The best predictive value for decline of FC was 88%. It is therefore reasonable to consider both patients with a normal FC level and those with an FC decline over 88% as responders. In addition, we were able to demonstrate that FC < 140 µg/g after stopping TNFα-blocking therapy indicates a relapse risk in asymptomatic patients already 6 months before the clinical or endoscopic relapse occurs. These findings are in line with the preliminary data from the STORI trial, revealing that FC begins to increase 4–6 months before the clinical relapse. Nonetheless, rather than setting one cut-off value for all IBD patients, one should take into consideration the behavioral and localization of the disease as well as the patient history.

While a single FC measurement has been shown to predict the relapse, De Vos and coworkers (2013) reported that two consecutive FC measurements > 300 mg/kg (µg/g) is more specific than a single measurement for predicting relapse in UC patients receiving IFX as a maintenance treatment. In addition, nearly 80% of the patients in sustained deep remission had at least
one FC measurement over 50 mg/kg (µg/g). These findings are similar to our study in which 65% of patients in stable remission had at least one FC measurement over 100 µg/g during the follow-up. De Vos and coworkers (2013) suggested that repeated measures of FC, rather than a single FC measurement, may help in adjusting therapy in UC patients before clinical symptoms emerge. Moreover, the results of our study indicated that repeatedly elevated FC levels predict clinical and/or endoscopic relapse, whereas mild, transient clinical symptoms seem to have no correlation with disease outcome. It is also noteworthy that inflammation is only one component of IBD. Symptomatic IBD patients who have normal biomarkers, should not simply be labeled as having IBS without further consideration. Patients may have symptoms due to noninflammatory mechanical disease such as bile salt malabsorption, fibrotic strictures, or adhesions. Nevertheless, patients with constantly elevated FC and ongoing clinical symptoms are the ones who benefit most from a close follow-up in clinical practice. More importantly, repeated measurements of FC appear to be a useful noninvasive tool for monitoring patients without endoscopy after cessation of TNFα-blocking therapy, as the STORI trial has already indicated. The optimal interval for measuring FC has not yet been determined. FC levels measured frequently, as often as every 4 weeks, could be the optimal approach for predicting relapse, but this warrants further research. Based on recent studies, restart of treatment would be possible only by documenting repeatedly elevated FC levels during follow-up; however, the endoscopic assessment of disease activity is currently recommended. Suggested follow-up strategies after cessation of TNFα-blocking therapy are presented in Figure 5.

4 Study limitations

These studies have some limitations. The patient groups were heterogeneous in all four studies, with both CD and UC patients included regardless of localization, behavior, or duration of disease, and therefore, the number of patients in the subgroups remained fairly low. In addition, the induction doses of both IFX and ADA varied, especially at the beginning of the study period, due to local variation in protocols. In Studies III and IV, only patients who had responded to TNF-blocking therapy sufficiently to continue with scheduled maintenance therapy for at least 11 months were included. This may overestimate the efficacy of biological therapies due to endoscopy-based case selection. Moreover, the study population in Study II included only those maintenance therapy patients who underwent ileocolonoscopy during the study period. Although the SES-CD is a validated score for assessment of endoscopic activity in CD, no validated cut-offs for endoscopic remission or relapse are available. For remission, we used the suggested SES-CD score of 0–2 published only as an abstract (Moskovitz et al. 2007). In patients with UC, an endoscopic Mayo score of 0–1 was defined as a remission, making it possible that some patients with UC had very mild endoscopic activity at baseline. However, we believe that inclusion of a low baseline FC value as
another criterion of remission in Studies II, III, and IV ruled out patients with notable inflammatory activity. Additionally, as the data were gathered retrospectively in Study II, clinical disease activity was assessed by the PGA rather than being based on any validated score. Furthermore, both clinical and endoscopic scoring was performed by several (albeit experienced) gastroenterologists, making some interobserver variability in the scoring system possible. Unfortunately, rescoring of the endoscopies was not possible. However, strength of our studies is that the disease activity surveillance included ileocolonoscopies with histological biopsy specimens, which were not performed in previous studies. During this study period analysis of FC samples was centralized at one laboratory, eliminating differences in the analysis process. In Study IV, more concise intervals for follow-up samples would have increased accuracy, but would have been more inconvenient for the patients. Thus, several patients failed to provide stool samples according to the study protocol. Despite the above-mentioned limitations, our studies reflect well the situation in clinical practice.
**Figure 5.** Treatment algorithm.
CONCLUSIONS

In the era of TNFα-blocking therapy, MH and even DR have been suggested as new therapeutic targets in both CD and UC. Recent studies have indicated that DR may be achieved in roughly half of IBD patients on TNFα-blocking maintenance therapy, resulting in more favorable treatment outcomes. However, considering the potential side-effects and economic issues associated with long-term immunosuppressive therapy with TNFα-blocking agents, the possibility of discontinuing therapy should be evaluated at least once after achieving DR.

At present, no widely accepted recommendations for discontinuing TNFα-blocking therapy are available. Based on the results of our study, only for about half of patients in DR were candidates for discontinuation of TNF-blocking medication for various reasons. Nevertheless, up to 67% of IBD patients in DR at the time of cessation of TNFα-blocking therapy remained in clinical remission during the 12-month follow-up, and moreover, the majority of these patients also remained in endoscopic remission. Therefore, withdrawal of therapy could be considered in IBD patients in DR even after one-year treatment. Reassuringly, in case of a relapse, the response to restart of TNFα-blocking therapy seems to be effective and well-tolerated.

To evaluate treatment response and possible relapse during maintenance therapy or after discontinuation of TNFα-blocking therapy, FC seems to be a reliable surrogate marker to replace endoscopic assessment. A normal FC after induction with TNFα-blocking agents predicts sustained remission in the majority of patients with active luminal disease receiving scheduled treatment. However, an objective cut-off value for treatment response and relapse risk is to some extent still undefined. To predict IBD relapse and identify patients requiring a close follow-up in clinical practice, FC seems to be a useful surrogate marker, as it increases and remains elevated as early as 6 months before symptomatic relapse. FC measurements should therefore be included in monitoring IBD patient’s treatment response in everyday clinical practice.
FINNISH SUMMARY

Yhteenveto

Tausta

Potilaat ja menetelmät

Tulokset
Päätelmät
Syvä remissio on saavutettavissa noin puolella anti-TNF-ylläpitohoitoa saavista potilaista. Biologinen lääkehoito on turvallista lopettaa vuoden kuluttua niiltä Crohnin tautia tai haavaista paksusuolitulehdusta sairastavilta potilailta, jotka ovat syvässä remissiossa. Hoidon uudelleen aloitus on sekä tehokasta että turvallista. Ulosten kalprotektiinin määritys biologisen lääkehoidon aikana sekä lääkehoidon lopettamisen jälkeen optimoi hoitoa ja sairauden seurantaa.
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