Univerzita Karlova v Praze
1. lékařská fakulta

Studijní program: Doktorský studijní program v biomedicíně
Studijní obor: Fyziologie a patofyziologie člověka

MUDr. Dana Ďuricová

Klinické a genetické prediktory lékové závislosti u idiopatických střevních zánětů

Clinical and genetic predictors of drug dependency in inflammatory bowel disease

Disertační práce

Školitel:
Prof. MUDr. Milan Lukáš, CSc.

Praha, 2012

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

PREFACE

This PhD thesis is based on the following papers:

I. Duricova D, Pedersen N, Lenicek M, Hradsky O, Bronsky J, Adamcova M, Elkjaer M, Andersen PS, Vitek L, Larsen K, Lukas M, Nevoral J, Wewer V, Munkholm P. Infliximab dependency in children with Crohn´s disease. Aliment Pharmacol Ther 2009; Apr1;29(7):792-9

II. Pedersen N, Duricova D, Lenicek M, Elkjaer M, Bortlik M, Andersen PS, Vitek L, Davidsen B, Wewer V, Lukas M, Munkholm P. Infliximab Dependency is Related to Decreased Surgical Rates in Adult Crohn´s Disease Patients. Eur J Gastroenterol Hepatol 2010; Oct;22(10):1196-203

III. Duricova D, Pedersen N, Elkjaer M, Slott Jensen KJ, Munkholm P. 5-Aminosalicylic Acid Dependency in Crohn´s Disease: A Danish Crohn Colitis Database Study. J Crohn Colitis 2010; Nov;4(5):575-81

IV. Duricova D, Pedersen N, Lenicek M, Jakobsen C, Lukas M, Wewer V, Munkholm P. The Clinical Implication of Drug Dependency in Children and Adults with Inflammatory Bowel Disease: A Review. J Crohn Colitis 2011; Apr;5(2):81-90

ABSTRACT IN CZECH

Léková závislost (dependence) jako specifický fenotyp idiopatických střevních zánětů (IBD), Crohnovy nemoci (CN) a ulcerózní kolitidy (UC), předurčuje prognózu onemocnění. Může být proto využit jako důležitý prognostický ukazatel při volbě optimálního terapeutického postupu. Dependence je dobře popsaná u IBD pacientů léčených kortikosteroidy a recentně byla také potvrzena u nemocných léčených infliximabem (IFX dependence). Cílem této práce bylo: 1) zhodnotit výskyt IFX dependence u dětských a dospělých pacientů s CN; dále identifikovat klinické a genetické prediktory odpovědi na IFX a zhodnotit vliv IFX dependence na frekvenci operací; 2) zhodnotit u pacientů s CN výsledky 5-ASA monoterapie s ohledem na výskyt 5-ASA dependence a identifikovat klinické prediktory odpovědi na 5-ASA.

Zjistili jsme, že 66% dětských a 29% dospělých pacientů s CN se stalo IFX dependentními. Vysoká frekvence u dětí je v souladu s dříve publikovanými prácemi, zatímco naše výsledky naznačují nižší výskyt IFX dependence u dospělé populace. Perianální onemocnění a nepřítomnost střevní operace před zahájením IFX byly identifikovány jako prediktory pro vznik IFX dependence u dětí. U dospělých pacientů, 2 genetické varianty *LTA* c.207 A>G a *CASP9* c.93 C>T byli asociovány s IFX odpovědí. Dětští i dospělí pacienti se setrvalou odpovědí na IFX a také ti se vzniklou IFX dependencí měli po zahájení terapie signifikantně nižší počet operací než non-respondenti na podávání IFX.

Třicet-jedna procent zkoumaných pacientů s CN bylo léčeno 5-ASA jako monoterapii. Z celkového počtu léčených 5-ASA vykazovalo 59% dlouhodobý efekt této terapie, přičemž 36% z nich dosáhlo setrvalou odpověď a u 23% pacientů došlo ke vzniku 5-ASA dependence. Ženy měly lepší dlouhodobou odpověď než muži, zatímco delší trvání nemoci bylo spojeno se vznikem 5-ASA dependence. Tento výsledek spolu s nálezem nejnižší frekvence střevních operací u 5-ASA dependentních pacientů zřejmě souvisí s velmi mírným typem onemocnění u této skupiny nemocných.

ABSTRACT IN ENGLISH

Drug dependency in inflammatory bowel disease (IBD), Crohn´s disease (CD) and ulcerative colitis (UC) is a specific disease phenotype which determines disease prognosis and hence may be used as a prognostic marker for treatment management. Drug dependency in IBD has been well described in corticosteroid treatment and recently also in infliximab (IFX) therapy. The aims of this thesis were: 1) to assess the occurrence of IFX dependency in paediatric and adult patients with CD; further to search for clinical and genetic predictors of IFX outcome and to evaluate the impact of IFX dependency on surgical rate; 2) to assess in CD patients the outcome of the first course of 5-ASA monotherapy with emphasis on 5-ASA dependency and to define clinical predictors of 5-ASA treatment outcome.

We found that 66% of children and 29% of adults with CD became IFX dependent. The high frequency in paediatrics is in agreement with previously published studies, while the finding in adult patients indicates a lower rate of IFX dependency in the only study to date. Perianal disease and no bowel surgery prior to IFX start were predictive of IFX dependency in paediatric patients. In adult cohort, 2 genetic variants *LTA* c.207 A>G and *CASP9* c.93 C>T were associated with IFX outcome, whereas no relevant clinical predictor was identified. We observed a significant decrease in surgical rates after IFX start both in prolonged responders and IFX dependent patients, in paediatrics and adults.

Thirty-one percent of studied CD patients had monotherapy with 5-ASA. Of the patients treated with 5-ASA 59% were shown to have a long-term benefit from 5-ASA as prolonged responders (36%) or 5-ASA dependent (23%). Female gender was found to be associated with a better long-term outcome, while longer disease duration was predicative of 5-ASA dependency. This together with an observation of the lowest surgical rate in 5-ASA dependent patients may suggest these individuals to have a very mild disease phenotype.

KEY WORDS IN CZECH

Infliximab dependence, mesalazin dependence, kortikosteroidní dependence, idiopatické střevní záněty, Crohnova nemoc, ulcerózní kolitida, průběh onemocnění, operace

KEY WORDS IN ENGLISH

Infliximab dependency, mesalazine dependency, corticosteroid dependency, inflammatory bowel disease, Crohn´s disease, ulcerative colitis, disease course, surgery

1   INTRODUCTION

Inflammatory bowel diseases (IBD) including Crohn´s disease (CD) and ulcerative colitis (UC) are chronic inflammatory disorders of gastrointestinal tract (1). In approximately one third of patients the disease is complicated by extraintestinal manifestations involving mainly joints, skin, eyes and liver (1). The peak age-interval at diagnosis is 16-35 years and six to seven percent of newly diagnosed patients are children below 15 years of age (2;3). Although both diagnoses share many similarities they represent two different entities as to pathologicoanatomic presentation, clinical manifestation and probably also etiopathogenesis (1).

CD is characterized by transmural, granulomatuos and segmental inflammation of gastrointestinal tract that may theoretically involve any part of the digestive tract from mouth to the anus (1). The most frequent localization of CD is terminal ileum and/or colon with approximately equal proportion of involvement (4). In about 10 to 15% of patients the disease is localized in proximal parts of the small bowel and the minority of patients has gastroduodenal or esophageal involvement (4). Special form of CD is perianal disease (about 30% of patients) presenting as perianal abscesses or fistulas. Clinical symptoms of CD are various and include abdominal pain of different quality, diarrhea, weight loss, fever and less frequently rectal bleeding (1).

UC is characterized by chronic inflammation of colonic mucosa only, involving rectum and spreading continuously as far as up to cecum (1). The mucosal changes may vary from mild findings including edema and loss of vascular pattern to severe changes of large ulcers (1). Based on the extent of inflamed colon UC may be classified as proctitis (involving only rectum), left-sided colitis (spreading up to splenic flexure) or extensive colitis (spreading beyond splenic flexure), with almost equal proportion of these localizations (5). In contrast to CD; clinical manifestation of UC is more uniform including diarrhea and/or tenesmus with or without rectal bleeding and mucus evacuation (1).

Both diseases are medically incurable and the goal of the current therapy is to induce and maintain long-standing remission. In majority of patients the disease course is characterized by periods of remission altered by episodes of active disease (relapses) of various length, frequency and severity (1). Nevertheless, about 10-15% of patients experience chronic continuously active disease despite the medical therapy (6). The disease location in CD seems to be more or less

stable; however the extent of UC tends to progress or regress over the time in a proportion of patients (7). Transmural nature of inflammation in CD may lead to local bowel complications such as strictures and/or bowel perforation in form of abscesses or fistulas and the frequency of these complications increases with the disease duration (8). As much as 70-80% of CD patients undergo intestinal surgery within 20 years after diagnosis, and about 30% of individuals require repetitive procedure (6;9;10). In UC, the cumulative probability of colectomy after 25 years of disease duration is 20-30%, and is the highest during the first year after diagnosis (4).

Although several clinical, genetic and serological markers have been identified to predict individual disease course, their use in daily clinical practice is very limited and the disease course and behaviour still remain somewhat unpredictable (11-13). Interestingly, self managed treatment with mesalazine and infliximab reflecting the individual disease course has been recently and successfully carried out in patients with IBD using the web based treatment systems (14;15). Future studies are needed to assess the potential of this new treatment approach to change the natural disease course.

1.1 Epidemiology

The highest incidence of IBD has been reported in western countries, particularly Scandinavian states, United Kingdom and North America (3;16-19). According to the latest epidemiological studies from the west Europe and North America performed after 1990 the incidence of CD ranges from 6.3 to $20.2/10^5$ inhabitants; corresponding incidence of UC varies from 3.9 to $17/10^5$ inhabitants (4). The incidence of CD and at least in some countries also of UC is still increasing, although a plateau or a decrease in incidence of UC had been observed in some European countries (20).

So far, the limited epidemiologic data from Eastern Europe or Asia reported considerably lower incidence of both diseases suggesting east- west gradient of incidence of IBD. Nevertheless the epidemiologic studies from these countries are mainly old of date and some of lower methodological quality. Furthermore the "eastern" life style has been changing towards the "western" one and the diagnostics of the disease have been also improving. Finally, some newer studies from east Europe indicate a relatively high incidence of IBD (4;20).

Due to these reasons the multicenter epidemiologic study investigating the incidence of IBD across European countries, Israel and one Asian country has been started (Burisch J et al, accepted at DDW congress San Diego 2012 for poster presentation). Based on the preliminary results the incidence of IBD overall, and UC and CD separately, in western countries is more than 2 times higher than the incidence in eastern countries (18.8. vs.7.7 for IBD; 10.1 vs. 4.4 for UC and 6.3 vs. 3.1 for CD). Hence, this finding confirms existing east-west gradient of the incidence of IBD. Moreover, it also demonstrates an increase in incidence of IBD in eastern countries compared to older reports.

Figure 1. East-West incidence gradient of IBD in 2010 (with permission from Johan Burisch)
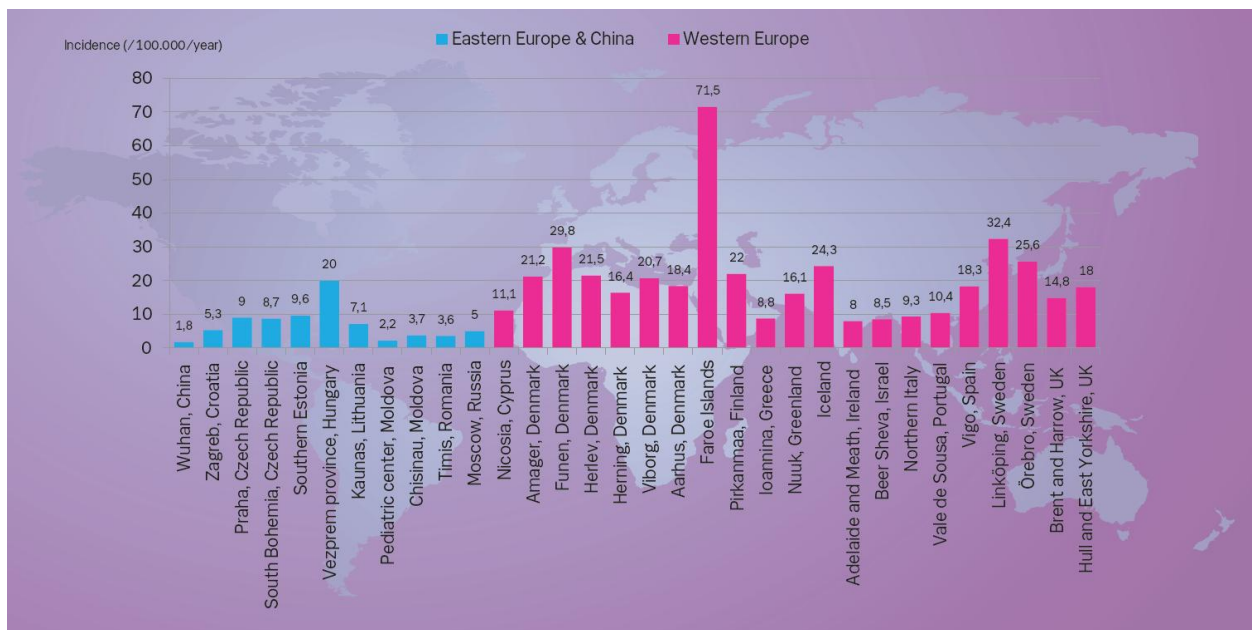
1.2 Etiopathogenesis of IBD

Although there is a big overlap between CD and UC, the diseases represent two different entities characterized also by different leading immunological profile. While CD seems to be mainly T helper (Th)1/Th17 cytokine mediated disease in which interleukin (IL)-12, IL-23 and interferon (IFN)-γ play a key role; UC is rather Th2 cytokine mediated disease with an IL-5 and IL-13 being the central cytokines (21). Both diseases then share common cytokines such as tumour

necrosis factor (TNF)-α, IL-6 or IL-1βF which are more or less associated with each of the disease and are produced mainly by innate immune cells (21;22).

Despite the long-term intensive research the origin and exact etiopathogenesis of either CD or UC is still unknown. Along with the intensive research providing constantly new knowledge, several theories have been proposed.

Mycobacterium avium subspecies *paratuberculosis* was thought to be an etiologic agent of CD (23). This belief arose from a link between a spontaneous granulomatous enterocolitis in cattle (Johne´s disease) caused by this microorganism and a later prove of mycobacterium in cultures from intestinal specimens, positive serology or molecular biologic techniques. Given the later negative studies on mycobacterium detection and lack of efficacy of anti-tuberculosis therapy in patients with CD, the proposed theory seems nowadays unlikely for most CD patients (23).

An increasing incidence of IBD paralleling improvements in hygiene and a higher incidence of IBD in developed than in developing countries lead later to a postulation of so called "hygiene hypothesis". The hypothesis was based on an assumption of a decreased microbial exposure in childhood resulting in an inappropriate development of immune system and subsequent abnormal response to potential pathogenic infectious agent in a later age, leading to development of IBD (24).

Currently, based on new emerging findings in the field of genetics, immunology and microbiology research, the most likely and the most accepted theory suggests that IBD results from pathological interaction between host immune system and bacterial intestinal flora in an individual with a certain genetic predisposition (23).

1.2.1 Genetics

Several indications such as increased risk of IBD among relatives, elevated risk of IBD in a population of Ashkenazi Jews and mainly twin studies showing higher concordance for IBD in mono- compared to dizygotic twins have demonstrated certain impact of genetics on pathogenesis of IBD (25-27). The genetic contribution seems to be higher in CD than in UC as apparent from twin studies with a higher concordance for CD than for UC (25;26).

To date, about 100 susceptibility loci have been identified to be significantly associated with IBD, encoding products which mediate variety of cell functions (microbial recognition,

lymphocyte activation, cytokine signalling and epithelial barrier function) (28). About one third of the susceptibility loci are associated with both diseases, the rest are specific either for CD or UC (29). Interestingly, there is a big genetic overlap between IBD and other immune and non-immune disease such ad type 1 and 2 diabetes, celiac disease, psoriasis, ankylosing spondylitis, etc (29).

Although approximately 100 of gene loci associated with IBD have been identified, their functional implication in IBD is largely unknown and a lot of loci seem to contribute only modestly to IBD risk (28).

In 2001, an association between polymorphisms in *NOD2* (*nucleotide binding oligomerization domain protein 2)* and CD was found, representing a landmark in IBD genetic research (30;31). So far, among the identified CD susceptible loci, *NOD2* polymorphisms seem to have the strongest association with the disease. In European population, individuals carrying at least one risk allele of *NOD2* were identified to have about 2-fold increase in risk for CD, whereas homozygotes or individuals with compound heterozygozity had up to 17-fold increase in risk for CD (32). Moreover, *NOD2* mutations have been consistently shown to be associated with ileal CD localization and stricturing/penetrating disease phenotype (32-34).

NOD2 is an intracellular receptor for microbial ligands which is broadly expressed in macrophages, dendritic cells, Paneth cells and to a lesser amount in intestinal epithelial cells (35). NOD2 is activated mainly by *N*-acetyl muramyl dipeptide (MDP), a bacterial component of peptidoglycan which is present in broad classes of bacteria (28;36;37). Stimulation of NOD2 by MDP results in activation of transcription factor nuclear factor-κB (NF-κB) pathway which is involved in expression of immune response genes in the gut (28;35).

Autophagy and genes encoding proteins involved in autophagy complex have been intensively studied during the last years. Autophagy is a process of cell self-degradation of damaged organelles and proteins and is important for the clearance of different types of pathogens as a part of innate immune system (28;29;38). Several polymorphisms in genes encoding proteins responsible for autophagy have been shown to be responsible for inefficient autophagic defence response to intracellular intestinal pathogens (38). Impairment in autophagy process is currently thought to play an important role in pathogenesis of CD. *ATG16L1* (*autophagy 16-like 1* polymorphism) encoding a protein involved in autophagy process, has been identified to be strongly associated with CD (39). *ATG16L1* is expressed in many cells, including Paneth cells

where it mediates exocytosis of secretory granules containing antimicrobial peptides (40). Other genes encoding proteins involved in autophagy discovered to be associated with CD are *IRGM* (immunity-related guanosine triphosphatase M) and *LRRK2* (leucine-rich repeat kinase 2) (41;42). Interestingly, a functional link between NOD2 and ATG16L1 in autophagy regulation has been reported (43;44).

Furthermore, several genes for cytokines and their receptors regulating T-cells have been studied in relation to IBD (28;29). Among others polymorphism in IL-23 receptor (IL23R) has been identified to be associated with IBD, and also other immune-mediated diseases (28). Molecules involved in IL-23 pathway seem to have a crucial role in maintaining intestinal immune homeostasis (29).

Several other genes encoding molecules assumed to be involved in the pathogenesis of IBD have been identified. Yet, either their functional role or their significance in the pathogenesis of the disease has to be determined.

1.2.2 Microbiota

The human intestine is estimated to contain 10^{13}-10^{14} microorganisms consisting of 15,000 to 36,000 individual species which can be grouped into 4 bacterial divisions: Firmicutes, Bacteroides, Proteobacteria and Actinobacteria (23;45). The concentration of microorganisms increases from proximal parts of gastrointestinal tract to colon with 10^2-10^3 aerobic organisms in gastrodudenum and 10^{11}-10^{12} mainly anaerobic organisms in cecum and colon (23).

There are several strong indications coming from animal models and human studies that intestinal microbiota play a central role in the pathogenesis of IBD. Most of germ-free animal models require colonisation with commensal bacteria in order to develop intestinal inflammation or immune activation (46;47). Increased levels of mucus- or mucosal-associated and translocated bacteria like adherent/invasive E coli strains in ileal mucosa of CD patients, have been found in IBD (48;49). In human studies, fecal stream diversion has been shown to either prevent recurrence of CD or treat active CD (50). Conversely, early recurrence of CD lesions has been observed after infusion of intestinal content to the excluded small bowel (51). There is also some evidence of beneficial effect of antibiotics and probiotics in the treatment of IBD (52-54) and a proportion of patients with IBD have serologic response towards several bacterial components

14

like ASCA (anti-*Saccharomyces cerevisiae* antibody), OmpC (antibodies to *E coli* outer-membrane porin C) Cbir 1 flagellin (*Clostridium species*) etc (55). Furthermore genetic polymorphisms involved in handling intestinal bacteria like NOD2 mutations or polymorphisms in autophagy genes support the theory of bacterial involvement in pathogenesis of IBD.

Dysbiosis

Intestinal bacterial flora in patients with IBD differs from that of healthy individuals with reduced diversity of commensal bacteria and increased numbers of mucosa-associated bacteria (48;56;57). Patients with IBD seem to have reduced number of bacteria with anti-inflammatory properties (species of Bacteroides and Firmicutes phyla) and/or increased number of pro-inflammatory acting microorganisms (Enterobacteriacae; mucolytic bacteria) (48). Furthermore, as already mentioned increased levels of mucosa-associated bacteria were found both in CD and UC compared to healthy controls (49;57). The clear origin of this dysbiosis in IBD patients is unknown and may arise either from host-mediated inflammatory response or from colonisation by enteric pathogens, or from combination of these (48). Hence, it is unknown if dysbiosis is a primary cause of IBD or just the consequence of the underlying disease (48).

1.2.3 Intestinal defence and immune-tolerance

In normal healthy individual defensive mechanisms of intestinal epithelial and immune cells are in homeostasis with commensal bacteria leading to an immune-tolerance of the gut microbiota. However, in patients with IBD several factors of the defense and immune tolerance mechanisms seem to be altered leading to disruption of the immune tolerance and inappropriate inflammatory response to antigens of autologous flora.

Intestinal defense

There are several components of defence mechanism including intestinal epithelial cells, innate and adaptive immune system and their co-operation, which are so far only partly understood. Recognition of intestinal bacteria and subsequent initiation of defense mechanisms is mediated via several pattern recognition receptors (PRRs), including toll-like receptors (TLR) and intracellular NOD-like receptor family, localized in epithelial and immune-competent cells

(22;38;58;59). Intestinal mucosal barrier with epithelial cells and mucus seem to play a central role in intestinal immune homeostasis. After activation of expressed PRRs intestinal epithelial cells produce cytokines and antimicrobial peptides and regulate intercellular interactions (38). Patients with CD, but also their relatives, have been found to have increased intestinal permeability, which may promote translocation of bacteria through the intestinal mucosa (60). An altered intestinal mucus barrier, particularly in mucus composition, has been demonstrated in individuals with UC (61).

Defensines are small cationic anti-microbial peptides produced mainly by Paneth cells and also other intestinal epithelial cells with a broad spectrum activity against bacteria, fungi and viruses which represent another part of innate immune defense mechanism of the gut (62-64). An altered secretion of these antimicrobial peptides has been described in animal models as well as in humans and linked to the polymorphisms in NOD2 receptor (63;64). Thus, it has been hypothesized that described association of ileal CD localization with NOD2 mutations might be at least partially attributed to an insufficient secretion of defensins, contributing to a breakdown of intestinal defence anti-microbial mechanisms (63;64).

Intestinal immune tolerance

An important feature of properly functioning intestinal immune system is not only efficient defence, but also mechanisms ensuring immune tolerance of commensal intestinal microbiota. Intact mucus layer and epithelial barrier limit penetration of bacteria into intestinal wall and thus minimize the exposure of microorganisms to cells of immune system (38). Furthermore several active mechanisms of down-regulation of innate and adaptive immune system exist. These include regulation of PRRs expression and responsiveness, secretion of inhibitory mediators and modulation of intracellular signalling pathways in distinct immune cells (38). IL-10 is an important anti-inflammatory cytokine mediating intestinal homeostasis among others by increasing expression of anti-inflammatory cytokines and inhibiting pro-inflammatory ones (28). Mice deficient in IL-10 have been shown to develop spontaneous colitis (65), furthermore a severe phenotype of CD has been described in patients with loss of IL-10 or its receptor function (28;29). Cells of innate immune system, such as lamina propria dendritic cells and macrophages, have been in centre of research attention regarding their role in IBD pathogenesis. A certain subset of lamina propria dendritic cells and macrophages seem to be important for differentiation

and maintenance of regulatory T-cells (Tregs) which are important in control of immune response (66;67). Alteration in these innate immune cells has been shown to decrease the number of Tregs resulting in an uncontrolled inflammation (38;66).

To date, far more other pathways of innate and adaptive immune systems have been identified to have a conceivable implication in the pathogenesis of IBD. Nevertheless, their functional link and mainly their importance in the pathogenesis of the disease have to be yet assessed. It is very likely that as IBD seem to be rather a heterogeneous disease; the relative importance of particular immune mechanisms may be different in different individuals. This may also be supported by differences in responses to particular medical therapies among individuals.

1.2.4 Environmental factors

Several environmental factors have been proposed to be associated with the risk of development of IBD. Some, like smoking have been consistently reported, others like diet are still discussed.

Smoking

Smoking is a well established factor having impact on development of IBD as well as on subsequent course of the disease. Smoking has been identified to be a risk factor for development of CD whereas there is an inverse relation with the risk of UC (68;69). Similarly, current smoking seems to deteriorate the disease course in patients with CD while its effect on UC is opposed (70). Furthermore, a higher prevalence of smokers among CD patients and a lower prevalence among UC individuals as compared to the general population have been observed (68;70). The exact pathophysiological mechanism of impact of smoking on IBD is unknown and proposed to be mediated by its several modulatory effects on humoral and cellular immune mechanisms (70).

Antibiotics

Antibiotics are frequently discussed external risk factor for development of IBD due to their gut microbiota modifying potential contributing to dysbiosis. Recently, an interesting Danish nationwide cohort study has been published on an association of antibiotic use in paediatrics and risk of childhood-onset IBD (71). The authors have found an increased risk for development of

CD in antibiotic users with a relative risk of 3.41 (95%CI: 1.45-8.02). The risk of CD was greatest in the first 3 months following antibiotic use, for penicillins, in age categories of 3-11 months and 2-3 years and in multiple antibiotic users (71). Interestingly, no risk for UC was observed.

Appendectomy

Appendectomy for appendicitis has been identified as a protective risk factor for development of UC (68;72-74). In a Swedish national registry based study patients who underwent appendectomy for appendicitis or mesenteric lymphadenitis had a significantly lower incidence of UC compared to individuals not having this surgery (incidence-rate ratio 0.73, 95%CI: 0.62-0.87 and 0.48, 95%CI: 0.27-0.83, respectively) (72). Interestingly, this decreased risk was observed only in individuals younger than 20 years of age. Furthermore appendectomies performed for nonspecific abdominal pain had no impact on UC development suggesting that appendicitis *per se* is the influencing factor (72). This observation was later supported by a nationwide Swedish-Danish cohort study showing that only appendectomies for appendicitis reduced the risk of UC onset (74). The etiopathogenetic link between appendicitis and risk of UC is unknown and hypothesized to be caused by different immune mechanisms involved in these two conditions (72). No or weak association with appendectomy has been observed in case of CD (75;76).

Diet

Several components of diet have been proposed to increase the risk (n-6 polyunsaturated fatty acids, animal protein intake, and dietary sugar intake) or to have a protective effect (n-3 polyunsaturated fatty acids) on development of either CD or UC (68;69). However, overall the results seem to be inconsistent, with a risk of methodological recall bias in some studies and mainly the causative role of these factors on IBD onset has to be further studied.

1.3 Therapy

IBD, as already mentioned, is a chronic lifelong disease characterized by periods of activity and remission. The goal of the current medical therapy is to induce remission of active disease and thereafter to maintain remission and prevent the relapse occurrence. Medications used for induction of remission are characterized by quick onset of anti-inflammatory efficacy and include mainly corticosteroids (either systemic or topical), 5-aminosalicylic acid (5-ASA) preparations, anti-tumour necrosis factor α (TNF α) monoclonal antibodies and cyclosporine (only in UC). Of them 5-ASA preparations and anti-TNFs are indicated also for maintenance therapy together with immunosuppressives - thiopurines and methotrexate (only in CD) which have a slow onset of their immunosuppressive activity. In addition to oral or parenteral preparations, local forms of corticosteroid and 5-ASA preparations are available in form of suppositories, enemas or foams and are prescribed mainly in UC for both active disease and maintenance of remission.

1.3.1 Corticosteroids

The first controlled trial demonstrating the efficacy of glucocorticosteroids in IBD was performed in 1955 by Truelove and colleagues using cortisone in patients with active UC (77). Subsequent introduction of corticosteroids into clinical practice represented a therapeutic landmark in IBD leading to improvement of patients' prognosis and decrease of previously high mortality. Corticosteroids are very potent anti-inflammatory agents with a response rate up to 90% of treated patients who are naive to this drug (78). Despite the favourable short-term effect their use in maintenance therapy is inappropriate due to non-efficacy in this indication and mainly because of multiple more or less severe adverse events (79).

Mechanism of action
The anti-inflammatory effect of glucocorticoids is mediated via binding to a cytoplasmatic glucocorticoid receptor which once the ligand is bound to it is moved to nucleus where it directly or indirectly regulates the target genes (80). Glucocorticoid –receptor complex binds to a specific DNA sequence (glucocorticoid response elements – GRE) which may result either in up-

regulation of anti-inflammatory genes, such as IL-10, annexin 1 or inhibitor of NF-κB, or downregulation of pro-inflammatory genes (81;82). Moreover, glucocorticoids may directly interact with NF-κB, activator protein (AP)-1 and other transcription factors and thus inhibit their functions (81). The majority of anti-inflammatory effects of glucocorticoids seem to be mediated through these inhibitory mechanisms (81). Furthermore, some effects of glucocorticoids are believed to be mediated by a distinct membrane receptor linked to a number of intracellular signalling pathways (80). Beside interaction of corticosteroids with inflammatory genes, they influence also expression of genes involved in metabolic processes resulting in some corticosteroid related side effects (81). Hence, novel glucocorticosteroids with strong anti-inflammatory properties but having considerably reduced risk of side effects have been under investigation.

1.3.2 5-aminosalicylic acid

Sulphasalazine, a combination of 5-ASA azo–bound to the antimicrobial acting sulphonamide - sulphapyridine was the first preparation of 5-ASA drugs used in IBD. The azo-bond is split by an azo reductase released by colonic bacteria, yielding 5-ASA and sulphapyridine. Later, it has been shown that the active therapeutic compound is represented by 5-ASA and sulphapyridine acts only as a carrier molecule which is responsible for most of the severe side effects of the drug (83-85). Thus in the 2nd half of 1980s new preparations including just 5-ASA (mesalazines) were introduced into clinical practice, having the same efficacy as sulphasalazine but considerably lower occurrence of adverse events (86-88). 5–ASA in an unprotected form is absorbed already in the proximal small intestine, thus precluding the drug to reach the colon in therapeutic concentrations (89). So far several formulations have been developed to delay the drug absorption classifying 5-ASA preparations into several groups (90;91):

1. Azo-bonded compounds – carrier molecule is linked to 5-ASA via diazo-bond as in sulphasalazine and releases 5-ASA in the colon by bacterial enzymatic activity.
2. pH-Dependent delayed release mesalazine - preparations are coated with Eudragit, a resin designed to dissolve at a certain pH (pH > 6 and 7 for Eudragit-S and Eudraget-L coated mesalazine, respectively) and release 5-ASA in the terminal ileum and colon.

3. Controlled-release mesalazine – contains microgranules of 5-ASA individually coated with semi-permeable ethylcellulose. The drug is steady released along the length of the intestine from the upper small bowel to the colon.

4. pH-Dependent multimatrix mesalazine (MMX) – the delivery system consists of hydrophilic and lipophilic matrices enclosed within a gastro-resistant, pH-dependent coating which delays release of 5-ASA at pH$\geq$7 (usually in the terminal ileum) and enables prolonged exposure of the colonic mucosa to 5-ASA.

Beside the oral formulations, 5-ASA is available also for rectal application in form of suppositories, foams or enemas.

Mechanism of action

5-ASA preparations, either oral or local, seem to work locally with the effectiveness related to the colonic mucosal/epithelial concentrations (92). The exact mechanism underlying its anti-inflammatory efficacy is not completely known, and seems to be heterogeneous.

Among the different effector mechanisms of 5-ASA belong modulation of arachidonic acid metabolism, modulation of production of inflammatory cytokines and inhibition of activation of NFκB pathway involved in expression of immune response genes in the gut (93). Furthermore 5-ASA acts as an antioxidant and free radical scavenger. Nevertheless, the main effect of 5-ASA seems to be as peroxisome proliferator –activated receptor –gamma (PPAR-γ) agonist (92;93). PPAR- γ is a transcription factor belonging to the nuclear receptor superfamily which mediates metabolic and nutritional signals into transcription events. PPAR- γ is expressed in high levels in colonic epithelial cells and plays a key role in bacteria-induced inflammation (93;94). The main anti-inflammatory effect of 5-ASA is currently thought to be exerted via direct activation of PPAR- γ. Furthermore, 5-ASA is believed to have chemoprophylactic effect on colonic neoplasia development which seems to be among others mediated through abovementioned mechanisms (93;95;96).

1.3.3 Anti-TNF α therapy

Infliximab (IFX) is a chimeric human/murine monoclonal antibody of IgG1 type against tumour necrosis factor α (TNFα) cytokine (97). IFX was the first monoclonal antibody shown to be effective in treatment of IBD and is used in this indication since 1998 and 2006 in CD and UC, respectively (97;98). Due to the chimeric origin of IFX which is probably responsible for increased immunogenicity resulting in allergic reactions and probably also contributing to loss of response, a fully human anti-TNFα IgG1 antibody –adalimumab- has been later developed (99). The drug was introduced into clinical practice for treatment of CD in 2006 and is awaited to be soon accepted also for UC (100;101). Both drugs are also affective in treatment of rheumatoid arthritis, ankylosing spondylitis and psoriasis (102-107). The 3rd anti-TNFα preparation used in IBD is certolizumab which is a PEGylated Fab´ fragment of humanized anti-TNFα monoclonal antibody (108). This drug is currently indicated for treatment of active CD and so far available only in USA, Switzerland and Russia. Another anti-TNFα preparation is etanercept which is a fusion protein consisting of the extracellular domain of TNF receptor and Fc domain of human IgG1 antibody (109). Its binding target beside TNFα contains also lymphotoxin α – a property not observed in other anti-TNF α preparations. Etanercept has been shown to be very efficacious in rheumatologic autoimmune diseases (109;110), however failed to prove its efficacy in treatment of IBD (111).

Tumour necrosis factor α

TNFα is an important proinflammatory mediator of innate and adaptive immunity and seems to play a key role in the pathogenesis of IBD and other autoimmune and non-autoimmune diseases such as rheumatoid arthritis and psoriasis, congestive heart failure, etc (112). Increased levels of this cytokine have been observed in patients suffering from these diseases (113;114). TNFα is produced by several cells such as macrophages, T lymphocytes, natural killer cells with the monocyto-macrophage population being the largest producers (115;116). The precursor form of TNFα is a transmembrane TNFα (mTNFα) expressed on activated macrophages, lymphocytes and other cells which is then cleaved by TNFα-converting enzyme to a soluble TNFα molecule (116;117). Both soluble and mTNFα have biologic efficacy mediated via type 1 and type 2 TNF receptor (TNFR1 and TNFR2) (116). Binding of soluble TNFα to TNFR1 has been thought to mediate the most biologic effects of TNFα. However, there seems to be an increasing evidence

of an important biologic role of mTNFα and TNFR2. Moreover, mTNFα not only acts as a ligand but also serves as a receptor with reverse signalling into cells expressing this molecule. This mechanism seems to be involved in the mode of action of anti-TNFα antibodies (116;117). The biologic effect of TNFα is pleiotropic, such as cytokine production, cell adhesion molecule expression, proliferation and apoptosis, depending on target cell type and amount and rapidity by which TNFα is produced (112;118;119). The exact mechanism by which TNFα may be involved in intestinal inflammation is unknown, most likely complex and exerted via several effects on immune and non-immune cells and production of molecules involved in immune mechanisms (112).

Mechanism of action of anti-TNFα antibodies

Soon after introduction of anti-TNFs, it has been obvious that blockade of either soluble or mTNFα *per se* cannot explain the pharmacodynamic effect of IFX (112). Since then several effector mechanisms of anti-TNFα antibodies have been proposed and so far confirm the complexity of mode of action of these drugs and to some extent elucidate the differences in anti-inflammatory efficacy observed among particular anti-TNFα preparations.

Complement dependent and antibody dependent cell cytotoxicity of mTNF expressing cells have been suggested as a potential mechanism of action of anti-TNFα antibodies (116;120;121). However, these findings were later not confirmed when activated normal peripheral blood mononuclear cells were used instead of transfected cell lines that may not correspond with the behaviour of normal human cells (122).

Later it has been shown that IFX induces apoptosis in peripheral monocytes from patients with steroid refractory CD (123). The apoptotic effect was found to be via binding of the Fab-portion of IFX to mTNFα and subsequent activation of the caspase-cascade independent of Fas/FasL mediated apoptosis (123). The same effect on peripheral blood monocytes was later demonstrated also for adalimumab, but not for etanercept (124). This failure of etanercept was speculated to be due to a lower avidity of entanercept to mTNFα, inability of cross-linking mTNFα and formation of less stable complexes with mTNFα compared to abovementioned anti-TNFs (124).

Another proposed mechanism of action of anti-TNFa antibodies has been via induction of apoptosis in activated T cells (125). Lamina propria T cells of patients with CD seem to be

resistant to apoptosis which probably plays an important role in controlling of lymphocyte proliferation (126;127). IFX has been shown to revert this defective T cell death and to induce apoptosis in mucosal and peripheral blood T cells via caspase signalling pathway independent of Fas/FasL mechanism (118;128). The proapoptotic effect of IFX has been demonstrated to be exerted through binding of IFX to mTNFα expressed in high amounts on activated human T cells (118;125).

A recent study from the Netherlands described an inhibitory effect of IFX and adalimumab on proliferation of activated T-cells (129). This effect was mediated via binding of anti-TNFs to both mTNFα and Fc receptor on regulatory macrophages. This concept stresses the dependence of immunosuppressive effect of anti-TNFs on presence of Fc region on these monoclonal antibodies which is responsible for differentiation of blood-derived monocytes to a certain subset of regulatory macrophages. Regulatory macrophages then display inhibitory effect on proliferation of activated T cells, and produce anti-inflammatory cytokine IL-10 (129). Etanercept and certolizumab failed to affect the proliferation of T cells in this study. The reason was likely in low binding efficacy to mTNFα of the first preparation and lack of Fc region of the later one. In contrast to previous studies, the authors did not prove proapoptotic effect of either IFX or adalimumab on T-cells. Their potential explanation was in the use of endogenous levels of mTNFα in contrast to overexpression and the use of highly viable peripheral blood lymphocytes in previous studies (129).

Another interesting study on mechanism of IFX action focusing on Tregs in patients with IBD was published by a German research group (130). Treg cells, as already mentioned, seem to have potent suppressive effect on proliferation of effector T cells and thus to contribute to suppression of inappropriate immune response which is thought to be one of the mechanisms leading to IBD (131;132). Decreased numbers of Treg cells have been described in active IBD correlating inversely with the disease activity (133;134). The authors have shown that apoptosis of Treg cells in patients with active IBD is strongly increased and that this may be reversed by IFX therapy in individuals responding to anti-TNFα preparation (130).

In conclusion, despite the intensive research in the field of anti-TNFs and emerging new findings the exact mechanism of action is largely unknown and still remains somewhat mysterious. It is likely that the combination of above mentioned and yet unknown mechanisms play a role in

efficacy of anti-TNFα preparations since the described experiments represent mainly in vitro single cells systems which may not properly reflect the complexity of the human organism.

2   DRUG DEPENDENCY - BACKGROUND

2.1   Drug dependency

IBD is a disease of various manifestations regarding disease localization, occurrence of complications and frequency of relapses (1). According to this, different behaviour patterns and disease courses have been identified classifying patients into several subtypes (6;7;135). Patients, however, differ also in response patterns to medications and beside the "classical" response/no response, long-term outcome „drug dependency" has been described in children and adults with IBD (78;136-139). The dependent patients represent a specific population of individuals who maintain remission while on the treatment, but relapse promptly after drug cessation or dose decrease. However, a quick restoration of remission repeating the pattern of former response is achieved and sustained when the therapy is re-introduced or dose increased.

2.2   Corticosteroid dependency

Drug dependency in IBD was for the first time described in corticosteroid treatment in 1994 in a population based study of newly diagnosed patients with CD from Copenhagen County (78). In an inception cohort of 109 CD patients naive to corticosteroids, 36% became steroid dependent after the 1st treatment course. Corticosteroid dependency was defined as relapse within 30 days after steroid discontinuation, or at dose decrease impeding discontinuation of steroids for more than 1 year (78).

Since then, frequency of corticosteroid dependency has been studied in several population-based and referral centre studies using modifications of the primary definition (137;138;140-143).

In 2006 the definition of corticosteroid dependency was implemented into European Crohn´s and Colitis Organisation (ECCO) guidelines for CD, later for UC in 2008 and also accepted by the Food and Drug Administration (FDA) agency as one of the end points for randomized controlled trials (144;145).

Population-based studies demonstrated that 28-36% of adult CD patients and 22% of UC patients became steroid dependent (78;137). Among paediatrics, 24-39% of CD children and 14-50% of

UC children were found to develop corticosteroid dependency (138;143;146;147). Similar results were found in studies of selected cohorts (140;142;148-150).

2.3   Infliximab dependency

Biological treatment, including infliximab (IFX), is nowadays considered the most potent anti-inflammatory therapy in IBD indicated in approximately 20-30% of patients who have severe and complicated disease course (79;88). The efficacy of IFX has been proven in both CD and UC, in adult as well in paediatric population, for induction and also maintenance therapy (97;98;151-154).

Soon, after introduction of IFX in to clinical practice, it was recognized that similarly to corticosteroid therapy, some patients were able to discontinue IFX and maintain long-term remission without further need of infusions, whereas a significant proportion of them relapsed shortly after drug withdrawal and required IFX re-introduction to sustain the remission. This response pattern was first described in 2006 in a retrospective paediatric study from Denmark and defined as IFX dependency (139). Children were considered IFX dependent if they relapsed within 90 days after IFX discontinuation but regained remission after its re-introduction. Of the 24 patients included 42% were found to be IFX dependent (139). Later, a national cohort of CD children from the Netherlands has been assessed for IFX dependency (136). Of 66 treated children, 56% were classified as IFX dependent (136).

So far, the use of IFX seems to be safe (152;155;156), nevertheless potential side effects such as opportunistic infections or allergic reactions might be very serious (157-161) and the consequences of the long-standing therapy are still being monitored. Moreover, the treatment is relatively expensive and the cost effectiveness is essential (162;163). Seen from these perspectives, early identification of prolonged responders and IFX dependent patients might be helpful in clinical practice when deciding for long-term treatment.

2.4   5-ASA dependency

5-ASA preparations are broadly used in mild to moderate UC showing efficacy in induction as well as in maintenance of remission (164;165). Contrary to this, the role of 5-ASA in CD is controversial. Several trials comparing effect of 5-ASA to placebo in CD patients have been done with divergent results (166-171). A meta-analysis investigating efficacy of 5-ASA in treatment of active CD and 2 meta-analyses focused on maintenance therapy failed to prove its efficacy (172-174). Consequently, the use of 5-ASA in CD was not more recommended (175). Nevertheless, the studies included were differently designed, the study population was quite heterogeneous and different drug formulations and dosages were used. Moreover, there is evidence from population based studies that a significant proportion of CD patients has a mild disease course (6;176). Furthermore, the newer 5-ASA preparations have a very good safety profile comparable to placebo (86).

The role of 5-ASA in CD is therefore still unclear and several factors indicate that there is a subgroup of CD patients who may benefit from 5-ASA therapy. Such individuals can be observed in clinical practice showing the similar dependent response pattern as seen in corticosteroid or IFX treatment.

3 HYPOTHESES

3.1 Paper I & II

The hypotheses of our study were:

1. IFX dependency occurs in approximately 50% of treated patients with an equal frequency in adult and paediatric patients

2. Clinical or genetic predictors of IFX dependency might better identify these patients who are in need of long-term IFX treatment

3. Contrary to corticosteroids, IFX dependency is associated with a favorable long-term prognosis having a low risk of surgery

3.2 Paper III

The hypotheses of our study were:

1. 5-ASA is efficacious therapy in a certain population of CD patients

2. Similar dependent pattern as in steroids or IFX is observed also in 5-ASA therapy

3. Predictors of long-term outcome of 5-ASA treatment may help to better select the individuals profiting from these preparations

4 AIMS

4.1 Paper I & II

The aims of our study were to:

1. Assess the frequency of IFX dependency in paediatric (Paper I) and adult (Paper II) patients with CD

2. Search for clinical and genetic predictors of IFX outcome

3. Evaluate the impact of IFX dependency on the surgical rate

4.2 Paper III

The aims of our study were to:

1. Assess the outcome of the first course of 5-ASA monotherapy with emphasis on 5-ASA dependency in patients with CD

2. Define conceivable clinical predictors of positive response to 5-ASA and mainly of 5-ASA dependency.

5 MATERIAL & METHODS

5.1 Paper I & II

5.1.1 Study population

Paediatric cohort

The study population included consecutive pediatric patients with CD treated with IFX who originated from: a) Danish Crohn Colitis Database (DCCD) treated at two departments of pediatrics in Denmark (Hvidovre and Odense University Hospital) from August 2000 until November 2006; b) the Czech Republic treated with IFX at two departments of pediatrics in Prague (Hospitals of the 1st and the 2nd Faculty of Medicine, Charles University) from October 2002 to June 2006. The diagnosis of CD of all patients was assessed according to the international diagnostic criteria (177).

Adult cohort

The study population comprised consecutive CD patients from the DCCD treated with IFX at Herlev and Slagelse University Hospitals between June 1999 and July 2006 and from the Czech Republic treated at Charles University Hospital in Prague between January 1999 and December 2005. The diagnosis of CD of all patients was assessed according to the international diagnostic criteria (144).

Controls

Healthy individuals, 182 from Denmark and 283 from the Czech Republic, were included as controls in the genotype-phenotype association study.

5.1.2 Infliximab therapy

IFX therapy was indicated for luminal disease (refractory or intolerant to conventional treatment, corticosteroid dependency) and perianal disease. The treatment strategy and cessation of the therapy were individualized with respect to patients' clinical condition and decision of treating physician. According to schedule of IFX administration, the treatment strategy was

retrospectively classified into 3 regimes: (1) induction therapy only (0, 2nd and 6th week); (2) induction therapy followed by scheduled maintenance therapy every 6 or 8 weeks and (3) on demand therapy when after the initial 1 to 3 infusions, the following infusions were given as non-scheduled in accordance to patient´s disease activity.

5.1.3 Data collection

Data on demographics and disease characteristics at IFX start including gender, age, disease localization and behaviour, disease duration, history of previous surgery; details on IFX therapy, concomitant medication and surgical treatment were retrieved from medical records.

5.1.4 Assessment of IFX outcome

Clinical outcome of IFX therapy was retrospectively assessed according to a modified phenotype model of IFX dependency developed and described previously (Table 1).

Table 1. Phenotype model of IFX dependency

Immediate outcome	Long-term outcome
30 days after the first infusion	3 months after last intended infusion
Complete response	*Prolonged response*
-Luminal disease: Total regression of symptoms: ≤2 stools/day (after surgery +2 stools). No blood, pus, mucus, abdominal pain and weight loss. -Perianal disease: Closure of all fistulas evaluated by thumb pressure or patients announcement of "no secretion".	Maintenance of complete or partial response.
Partial response	*IFX dependency*
-Luminal disease: Regression of symptoms: ≤4 stools/day (after surgery +2 stools); blood, pus, mucus, abdominal pain less than daily, no fever and weight loss. -Perianal disease: Reduced secretion or discomfort from fistulas or closure of one or some of the fistulas.	Relapse requiring repeated infusions to regain the complete or partial response; patients who needed IFX therapy for more than 12 months to sustain response were also considered IFX dependent.
No response	*No response*
-Luminal/perianal disease: No regression of symptoms with a need to shift to another immunomodulator and/or surgery within 3months after initiation of IFX.	No improvement or worsening of symptoms with a need to shift from IFX treatment to another immunomodulator and/or surgery.

The last intended infusion meant the primary decision of treating clinician to stop IFX therapy. Since the number of infusions given prior to intended IFX stop differed among the patients the long-term outcome was evaluated irrespective of the prior treatment length.

Immunomodulator was defined as corticosteroids, thiopurines, methotrexate and other biological drugs.

Surgery was classified as intestinal (resection, strictureplasty, colectomy) or perianal (incision of abscess, fistulotomy, advancement flap). Incision of perianal abscess as a possible consequence of healing process during IFX treatment was not considered as surgery.

If the interval between IFX infusions was >1 year, the treatment was considered as the 2nd, the 3rd etc. course. Only the 1st treatment course was analyzed in this study.

5.1.5   Genetic polymorphisms

Following candidate variants were selected as conceivable predictors of IFX outcome: *TNF* c.-1037 C>T, *TNF c.*-488 A>G, *CASP9* c.93 C>T, *LTA* c.207 A>G and *FASLG c.*-844 C>T.

Genomic DNA was isolated from peripheral blood by routine salting out procedure (Czech Republic) or from buccal swab (Denmark) using Qiagen DNA purification Kit (Qiagen, Hilden, Germany). All polymorphisms were typed using PCR- restriction fragment length polymorphism as described in Paper I.

To ensure consistency between runs, samples of known genotypes were repeated in every analysis. DNA samples were analysed under numeric codes, and genotype-phenotype matching was done at the end of the study. Genotypes of controls were in Hardy-Weinberg equilibrium.

Czech and Danish cohorts were analyzed separately due to significant differences of *TNF* c.-1037 C>T, *TNF c.*-488 A>G, *LTA* c.207 A>G and *FASLG c.*-844 C>T variants in general populations.

5.1.6   Statistical analysis

Empirical transition probabilities from immediate outcome to long-term outcome were calculated. Univariate logistic analyses were carried out analyzing the probability of being prolonged responder and/or IFX dependent at long-term outcome. Fisher exact test was used

when appropriate. Chi-square test and Fisher exact test were used to compare allelic and genotype frequencies and to analyze an association of present variants with response to IFX. Patients´ clinical and demographic characteristics were compared by Mann-Whitney test or Chi-square test. Kaplan-Meier curves with accompanying log-rank test were done for time until surgery. A significance level of 5% was chosen.

5.2 Paper III

5.2.1 Study population

The study population consisted of CD patients from DCCD treated at Herlev University Hospital in a time period from 1953 to 2007. From May 2007 to April 2008 the medical records of all CD patients were scrutinized to abstract information on treatment with per oral and topical 5-ASA preparations. Patients fulfilling the following inclusion criterion were included into data analyses: „Relapse of the disease with the intention to treat only with 5-ASA preparations during the disease course".

5.2.2 Data collection

Data on age, gender, disease duration, previous surgery, disease behaviour and localization at the start of 5-ASA monotherapy and the details on 5-ASA treatment were retrieved from medical files.

5.2.3 Assessment of 5-ASA response

Response to 5-ASA treatment was evaluated retrospectively according to a developed phenotype model of 5-ASA dependency (Figure 2).

Immediate outcome: 30 days after the start of 5-ASA therapy
1. *Complete response:* Total regression of clinical symptoms
2. *Partial response:* Improvement of clinical symptoms
3. *No response*: No regression of symptoms with a need to shift from 5-ASA to immunomodulator and/or surgery

Long-term outcome: irrespective of the length of the treatment
1. *Prolonged response:* Still in complete/partial response one year after induction of response, either on the initial/reduced dose of 5-ASA or after 5-ASA discontinuance

2. *5-ASA dependency:* Relapse within one year after 5-ASA treatment cessation and regain of complete/partial response after 5-ASA re-introduction or relapse on a dose reduction or stable dose requiring dose escalation to regain complete/partial response

3. *No response:* No regression of symptoms with a need to shift from 5-ASA treatment to immunomodulator therapy and/or surgery

Figure 2. Clarification of the definitions of the long-term response to 5-ASA

Prolonged response
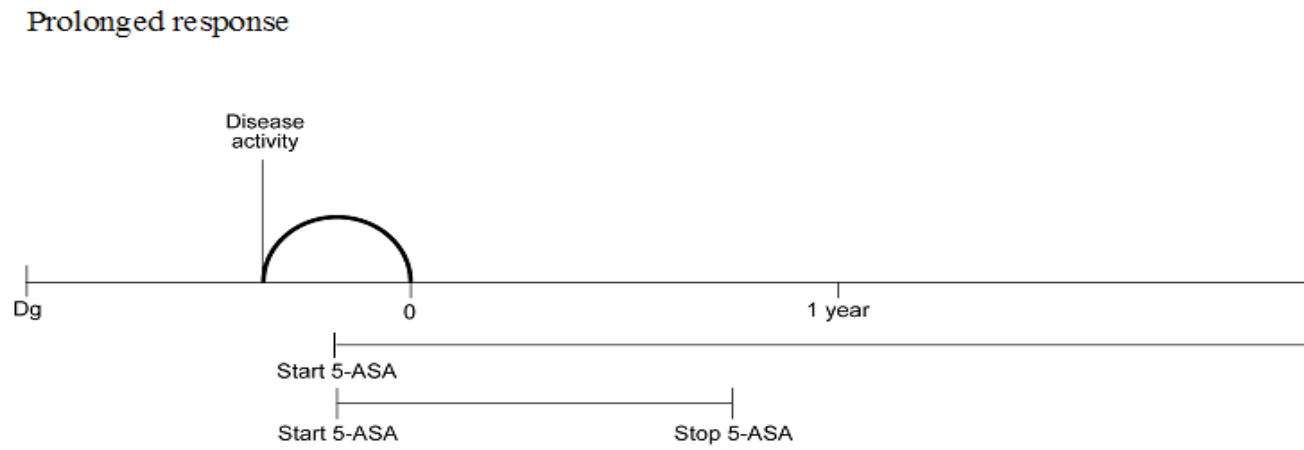
5-ASA dependency
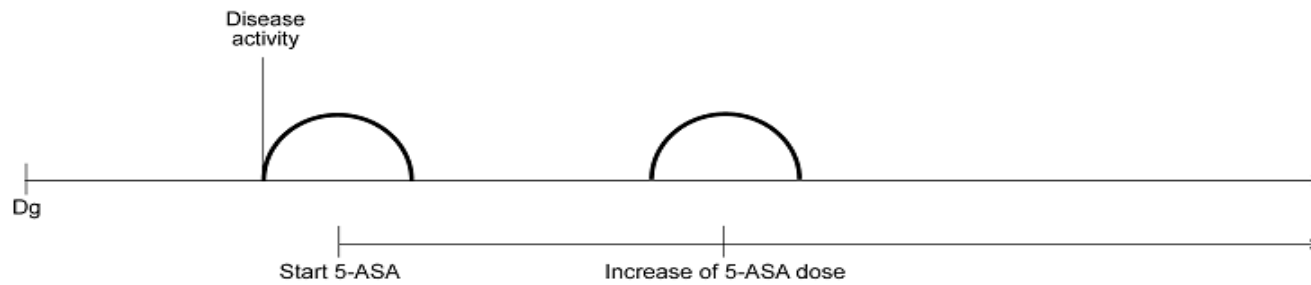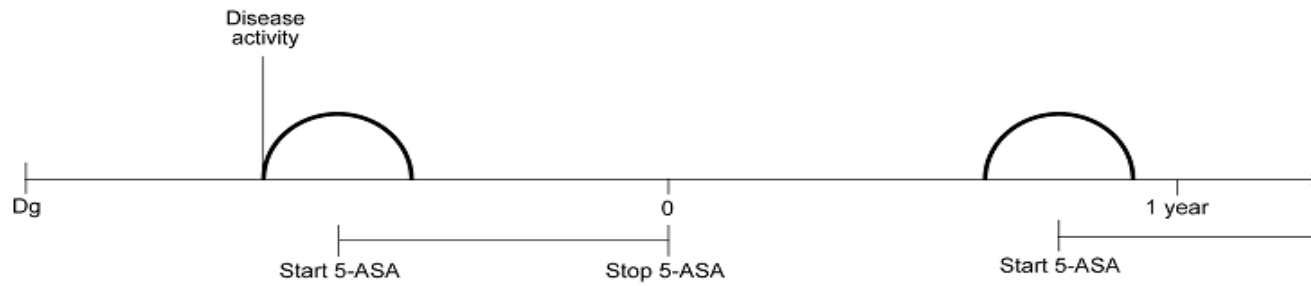
Dg, diagnosis

Immunomodulator therapy included corticosteroids (intravenous, per oral, enemas/foams), thiopurines, methotrexate and biological drugs. Surgery was defined as intestinal (resection, strictureplasty, colectomy) or perianal (incision of abscess, seton, fistulotomy).

A new relapse with the intention to treat only with 5-ASA >1 year after the previous 5-ASA course or after the immunomodulator treatment or surgery was considered as the 2nd, 3rd etc. course. Only the 1st treatment course was analyzed.

5.2.4   Statistical analysis

Logistic regression analyses were performed in order to investigate which disease characteristics were predictable for a certain treatment response. Kaplan-Meier curve with accompanying log-rank test was done for cumulative probability of surgery. A significance level of 5% was chosen.

6 RESULTS

6.1 Paper I

A total of 82 children with CD were included, 41 from Denmark and 41 from the Czech Republic. Baseline demographic and clinical characteristics are outlined in Table 2. The median (range) follow-up after start of IFX therapy was 45 (3-75) and 21 (6-50) months for Danish and Czech children, respectively.

Table 2. Patients´ demographic and clinical characteristics at start of IFX therapy

	Danish (n=41)	Czech (n=41)	p
Median age (range)	14 (9-18)	15 (8-18)	0.58
Female (%)	23 (56%)	19 (46%)	0.38
Median disease duration (yr)	2 (0-7)	2 (0-6)	0.50
Disease localization (%)			0.23
Ileum (L1)	0	3 (7)	
Colon (L2)	15 (37)	10 (24)	
Ileo-colon (L3)	17 (41)	20 (49)	
Upper disease +/- L1,2,3	9 (22)	8 (20)	
Disease behaviour (%)			0.01
Inflammatory (B1)	24 (58)	11 (27)	
Stricturing+penetrating (B2+3)	6 (15)	14 (34)	
Perianal+ B1/2+3	11 (27)	16 (39)	
Prior intestinal surgery (%)	8 (20)	3 (7)	0.19
Concomitant immunosuppressive therapy (thiopurines /methotrexate) (%)	36 (88)	39 (95)	0.49

IFX was indicated for luminal and perianal disease in 62 and 20 children, respectively. Twelve children received only induction infusions, 38 children were treated with induction infusions followed by maintenance therapy (median: 10 infusions; range: 5-21) and 31 received IFX on demand (median: 6; range: 1-27) (Table 3). In 2 patients on maintenance therapy, the dose of IFX was increased to 10 mg/kg later in the treatment course due to loss of efficacy. One patient was lost from the follow-up.

Table 3. Details on IFX therapy in paediatric patients with CD with respect to country of origin

		Danish (n=41)	Czech (n=41)	p
Indication to IFX	- Luminal disease	35 (85)	27 (66)	0.04
	- Perianal disease	6 (15)	14 (34)	
Treatment regime	- Only induction	6 (15)	6 (15)	0.04
	- Maintenance	14 (34)	24 (60)	
	- On demand	21 (51)	10 (25)	

6.1.1   Outcome of infliximab treatment

The immediate response was obtained in 77 (94%) children of whom 65 (79%) obtained complete and 12 (15%) partial response. In long-term outcome 18 (22%) patients achieved prolonged response, 53 (66%) developed IFX dependency and 10 (12%) patients were non-responders. The median number of infusions given were 3 (range: 1-6) to prolonged responders, 10 (5-27) to IFX dependent children and 2 (2-3) to non-responders.

Transition probabilities from immediate to long-term outcome are shown in Figure 3.

Figure 3. Transition probabilities from immediate to long term outcome with respect to immediate response
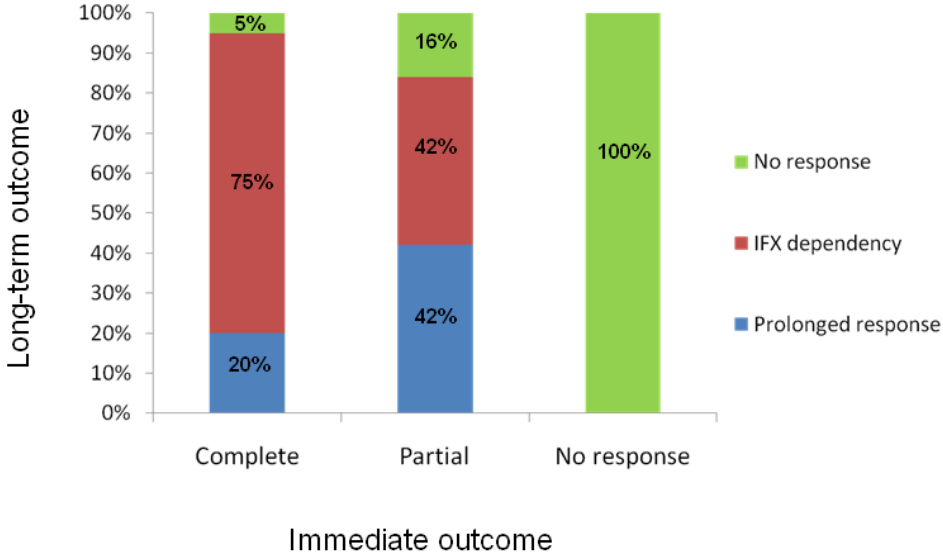
6.1.2 Clinical predictors

Inflammatory disease behavior (OR ∞, 95%CI: 3.23-∞) was predicative of prolonged response or IFX dependency while prior intestinal surgery decreased the probability of this response (OR 0.05, 95%CI: 0.01-0.32).

Regarding IFX dependency, complete immediate response (OR 3.9, 95%CI: 1.13-13.22), perianal disease (OR 5.34, 95%CI: 1.24-22.55) and no prior intestinal surgery (OR 6.7, 95%CI: 1.67-26.61) were associated with IFX dependent response (Table 4).

Table 4. Clinical predictors of long-term outcome in children with immediate complete or partial response to IFX (n=76)

	Prolonged response & IFX dependency	IFX dependency
Predictor variable	OR (95%CI)	OR (95%CI)
Czech vs. Danish	2.24 (0.37-13.53)	1.08 (0.40 - 2.90)
Male gender	1.00 (0.18-5.48)	1.28 (0.47-3.43)
Age at IFX start ≥17 yr vs. ≤16 yr	0.33 (0.05-2.14)	0.91 (0.24-3.47)
Disease duration >2 yr vs. ≤2 yr	3.33 (0.35-31.46)	0.63 (0.23-1.71)
Disease localization -Colonic vs. Ileal -Ileocolonic vs. Ileal - Upper vs. Ileal	(-) 0 (0-18.41) 0 (0-7.51)	1.21 (0.27-5.40) 0.45 (0.11-1.86) 1.09 (0.15- 8.12)
Behaviour - Non-inflammatory vs. Inflammatory	0 (0 – 0.31)*	0.39 (0.11-1.32)
Prior intestinal surgery	0.05 (0.01 - 0.32)*	0.15 (0.03-0.66)*
Indication to IFX - Luminal vs. Perianal disease	0 (0-2.53)	0.19 (0.04- 0.92)*
Concomitant immunosuppressants	3.30 (0.29 - 37.11)	1.48 (0.22- 9.89)
Immediate outcome -Partial vs. Complete	0.33 (0.05 - 2.14)	0.26 (0.07 - 0.95)*

OR, odds ratio; CI, confidence interval; * statistically significant results

6.1.3 Genetic predictors

No association was found between studied polymorphisms and IFX outcome (Table 5).

Table 5. Minor allele frequency (%) vs. IFX outcome in children with CD

| Variant | Immediate response (n=16) | | Long-term response (n=15) | | | | Immediate response (n=36) | | Long-term response (n=34) | | |
	CR+PR (n=15)	NR (n=1)	PRO+ID (n=14)	NR (n=1)	ID (n=11)	PRO+ NR (n=4)	CR+PR (n=35)	NR (n=1)	PRO (n=9)	ID (n=25)	NR (n=0)
FASLG c.-844 C>T	37	50	56	50	32	50	41	100	22	48	-
CASP9 c.93 C>T	17	50	18	0	18	12.5	26	50	28	26	-
LTA c.207 A>G	30	50	32	0	32	25	36	50	28	38	-
TNF c.-488 A>G	13	50	17	0	18	0	13	50	11	14	-
TNF c.-1037 C>T	0	0	0	0	0	0	11	0	11	12	-

The two main column groups are headed **Denmark** and **Czech Republic**.

CR, complete response; PR, partial response; NR, no response; PRO, prolonged response; ID, IFX dependency

6.1.4 Surgery

The cumulative probability of surgery is outlined in Figure 4. Fifty months after the start of IFX therapy the risk of intestinal surgery was 10% in IFX dependent patients, 30% in prolonged responders and 70% in non-responders (p=0.0002). IFX dependent children had significantly lower surgery rate compared to those with prolonged response (p=0.036).

Figure 4. Proportion of CD patients without intestinal surgery after the start of IFX therapy
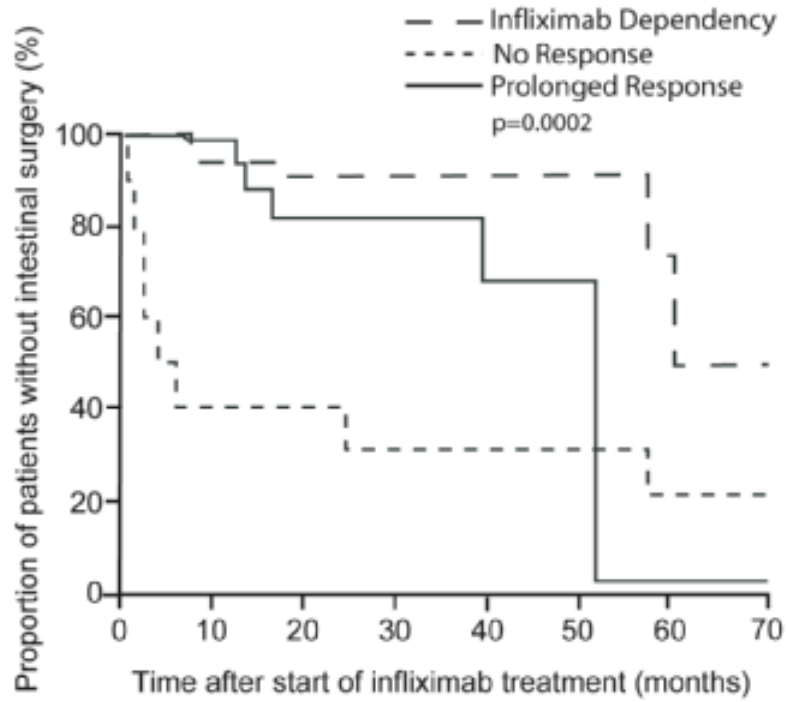
Number of patients at risk

Prolonged Response	17	15	11	8	6	2	0	0
Infliximab Dependency	53	47	29	18	10	7	3	0
No Response	10	4	4	3	3	3	1	0

6.2   Paper II

The study cohort comprised 132 CD patients from Denmark and 113 from the Czech Republic with the median (range) follow-up of 37.5 (6-92) and 38 (13-89) months respectively. The baseline demographic and clinical characteristics are presented in Table 6.

Table 6. Patients´ demographic and clinical characteristics at start of IFX therapy

	Danish (n=132)	Czech (n=113)	p
Medina age (range)	34 (15-66)	31 (16-67)	0.08
Female (%)	72 (58%)	60 (53%)	0.48
Median disease duration in years (range)	6 (0-33)	5 (0-39)	0.50
Disease localization (%)			0.01
Ileum (L1)	18 (14)	12 (11)	
Colon (L2)	47 (35)	35 (31)	
Ileo-colon (L3)	63 (48)	49 (43)	
Upper disease +/- L1,2,3	4 (22)	17 (15)	
Disease behaviour (%)			0.07
Inflammatory (B1)	48 (36)	26 (23)	
Stricturing+penetrating (B2+3)	18 (14)	20 (18)	
Perianal+ B1/2+3	66 (50)	67 (59)	
Prior intestinal surgery (%)	56 (42)	64 (57)	0.03
Concomitant immunosuppressive therapy (thiopurines/methotrexate) (%)	114 (86)	79 (70)	0.002

The indication for IFX therapy was luminal and perianal disease in 132 and 114 patients, respectively. Induction therapy only was given to 47% of patients, induction followed by maintenance therapy to 14% of patients (median 8 infusions, range: 4-17) and on demand therapy to 39% of patients (median 4 infusions, range: 1-28) (Table 7). The interval between infusions given on demand was 6-50 weeks. Two patients needed to increase the dose of IFX to 10 mg/kg during the treatment course.

Table 7. Details on IFX therapy in patients with CD with respect to country of origin

		Danish (n=132)	Czech (n=113)	p
Indication to IFX	- Luminal disease	77 (58)	54 (48)	0.10
	- Perianal disease	55 (42)	59 (52)	
Treatment regime	- Only induction	31 (23)	84 (74)	<0.001
	- Maintenance	26 (20)	8 (7)	
	- On demand	75 (57)	21 (19)	

6.2.1 Outcome of IFX treatment

Immediate response was observed in 210 (86%) patients: 136 (56 %) patients achieved complete, 74 (30%) partial and 35 (14 %) had no response.

In long-term outcome 114 (47%) patients obtained prolonged response, 71 (29%) developed IFX dependency and 60 (24%) had no response. The median number of infusions was 3 (range: 1-7) in prolonged responders, 9 (3-28) in IFX dependent patients and 3 (1-7) in non-responders.

The analysis of transition probabilities from immediate to long-term outcome with respect to immediate response is outlined in Figure 5.

Figure 5. Transition probabilities from immediate to long term-outcome
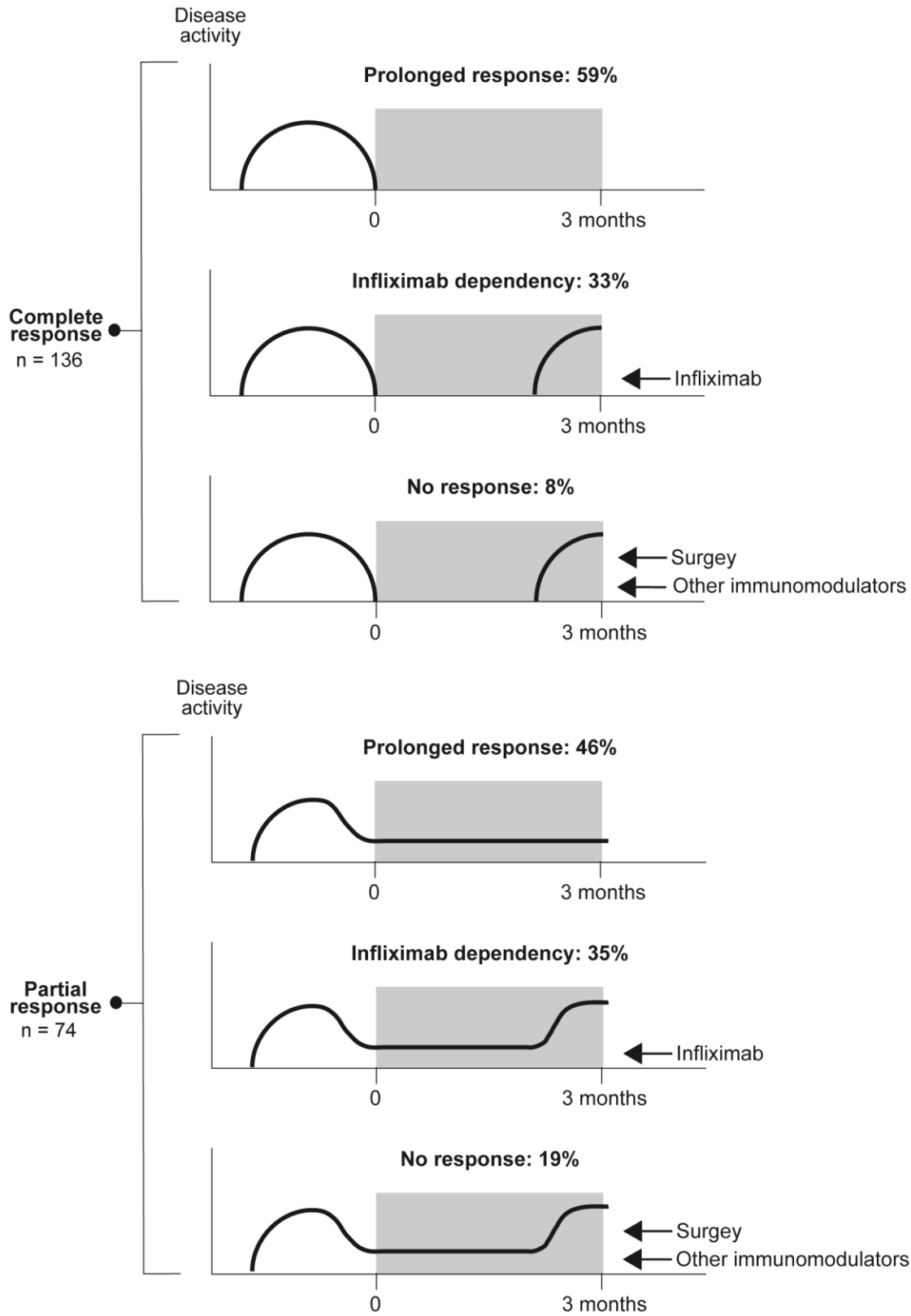
6.2.2 Clinical predictors

Patients with an immediate complete response were more likely to sustain prolonged response or develop IFX dependency than those with partial response (OR 2.65; 95%CI: 1.54 – 6.09). Prior intestinal surgery was shown to be negatively associated with IFX dependent phenotype (OR 0.45; 95%CI: 0.24-0.82). Czech patients developed significantly less IFX dependent response than Danish patients (OR 0.37; 95%CI: 0.20-0.69) (Table 8).

Table 8. Clinical predictors of long-term outcome in CD patients with immediate complete or partial response to IFX (n=210)

	Prolonged response & IFX dependency	IFX dependency
Predictor factor	OR (95%CI)	OR (95%CI)
Czech vs. Danish	0.78 (0.33 – 1.84)	0.37 (0.20 – 0.70)*
Male gender	1.19 (0.50 – 2.85)	0.76 (0.42-1.38)
Age at IFX start >40 yr vs. ≤40 yr	0.98 (0.38 - 2.53)	0.84 (0.43-1.63)
Disease duration >2 yr vs. ≤2 yr	0.99 (0.39 – 2.57)	1.11 (0.57-2.13)
Disease localization -Colonic vs. Ileal -Ileocolonic vs. Ileal - Upper vs. Ileal	1.04 (0.29-3.76) 1.64 (0.45-5.972) 1.48 (0.23-9.37)	1.83 (0.64-5.24) 1.81 (0.65-5.02) 3.15 (0.85- 11.61)
Behaviour - Non-inflammatory vs. Inflammatory	0.47 (0.14-1.65)	0.57 (0.23-1.44)
Prior intestinal surgery	0.54 (0.23-1.29)	0.45 (0.24-0.82)*
Indication to IFX - Luminal vs. Perianal disease	0.85 (0.36-2.00)	1.51 (0.84- 2.73)
Concomitant immunosuppressants	1.26 (0.46 - 3.45)	1.41 (0.66- 3.00)
Immediate outcome -Partial vs. Complete	0.38 (0.16-0.90)*	1.10 (0.60 – 2.01)

OR, odds ratio; CI, confidence interval; * statistically significant results

6.2.3 Genetic predictors

Two variants were found to be associated with IFX outcome. Danish patients carrying at least one G allele of *LTA* c.207 A>G variant were more likely to become prolonged responders or IFX dependent compared to those homozygous for A allele (93% vs. 69%, respectively; OR 6.04, 95%CI: 1.48-25.26). In Czech patients, IFX dependent phenotype was associated with the presence of at least one T allele of *CASP9* c.93 *C>T* variant (CT/TT vs. *CC*: 37% vs. 14%, OR 3.55, 95%CI: 1.21-10.37) (Table 9).

Table 9. Genotype frequencies vs. IFX outcome in patients with CD

Variant	Genotype	Denmark					Czech Republic				
		Immediate outcome (n=100)		Long-term outcome (n=86)			Immediate outcome (n=90)		Long-term outcome (n=77)		
		CR+PR (n=86)	NR (n=14)	PRO+ID (n=77)	NR (n=9)	ID (n=39)	CR+PR (n=77)	NR (n=13)	PRO+ID (n=58)	NR (n=10)	ID (n=19)
FASLG c.-844 C>T	CC	29	7	26	3	9	35	8	28	7	6
	CT	39	4	34	5	20	35	5	32	3	10
	TT	18	3	17	1	10	7	0	7	0	3
CASP9 c.93 C>T	CC	53	6	45	8	20	42	7	36	6	**6 ***
	CT	27	7	26	1	15	28	6	24	4	**11**
	TT	6	1	6	0	4	7	0	7	0	**2**
LTA c.207 A>G	AA	13	3	**9***	4	7	5	2	4	1	1
	AG	36	5	**33**	3	16	33	6	30	3	6
	GG	37	6	**35**	2	16	39	5	33	6	12
TNF c.-488 A>G	AA	3	0	2	1	1	2	0	2	0	0
	AG	26	5	23	3	14	15	2	13	2	3
	GG	57	9	52	5	24	60	11	52	8	16
TNF c.-1037 C>T	CC	72	10	63	9	32	57	13	50	7	14
	CT	14	4	14	0	7	19	0	16	3	5
	TT	0	0	0	0	0	1	0	1	0	0

*statistically significant result

CR, complete response; PR, partial response; NR, no response; PRO, prolonged response; ID, IFX dependency

6.2.4 Surgery

The cumulative rate of intestinal surgery 40 months after the start of IFX was 20% in prolonged responders, 23% in IFX dependent patients and 76% in non-responders (p<0.001) (Figure 6). Danish patients had significantly higher risk of surgery than the Czech patients (p=0.04) (Figure 7).

Figure 6. Proportion of CD patients without intestinal surgery after the start of IFX therapy
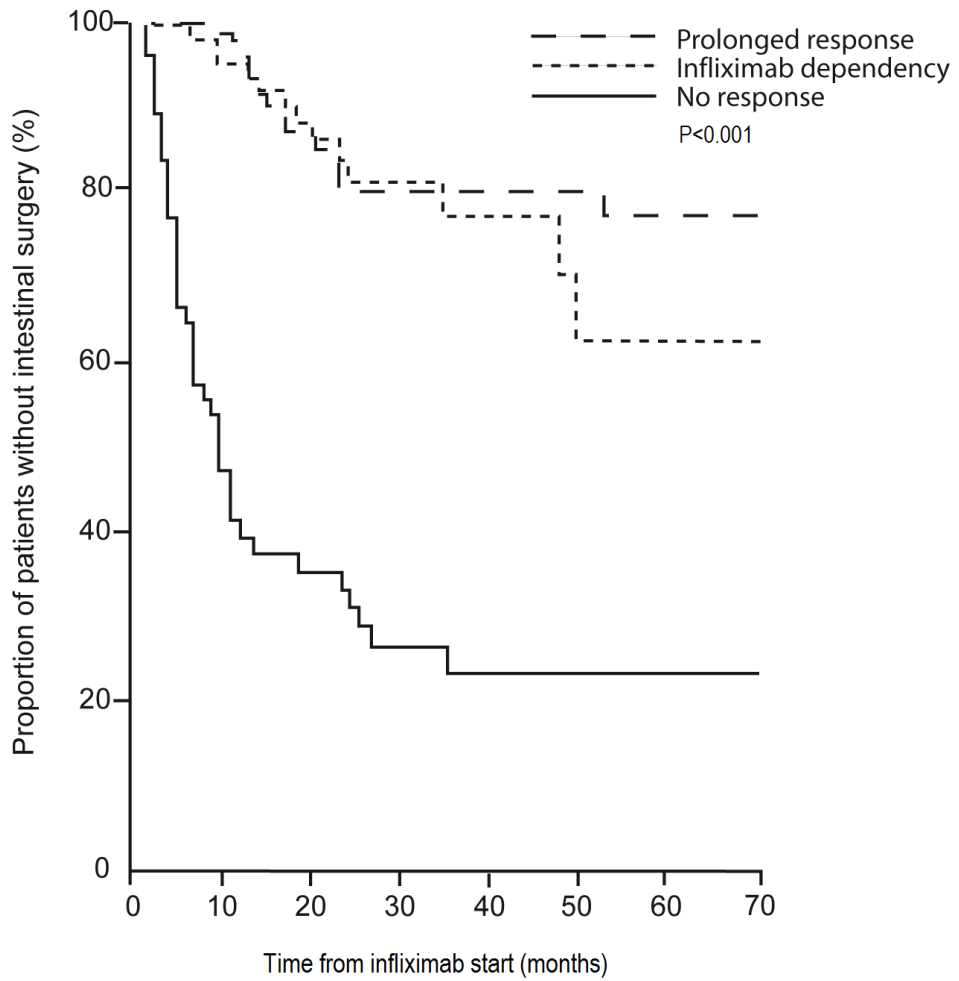
Number of patients at risk

Prolonged response	113	110	86	61	41	36	25	9
Infliximab dependency	69	62	46	26	18	10	7	4
No response	58	31	18	12	9	7	5	0

Figure 7. Cumulative probability of surgery after IFX start with respect to country of origin
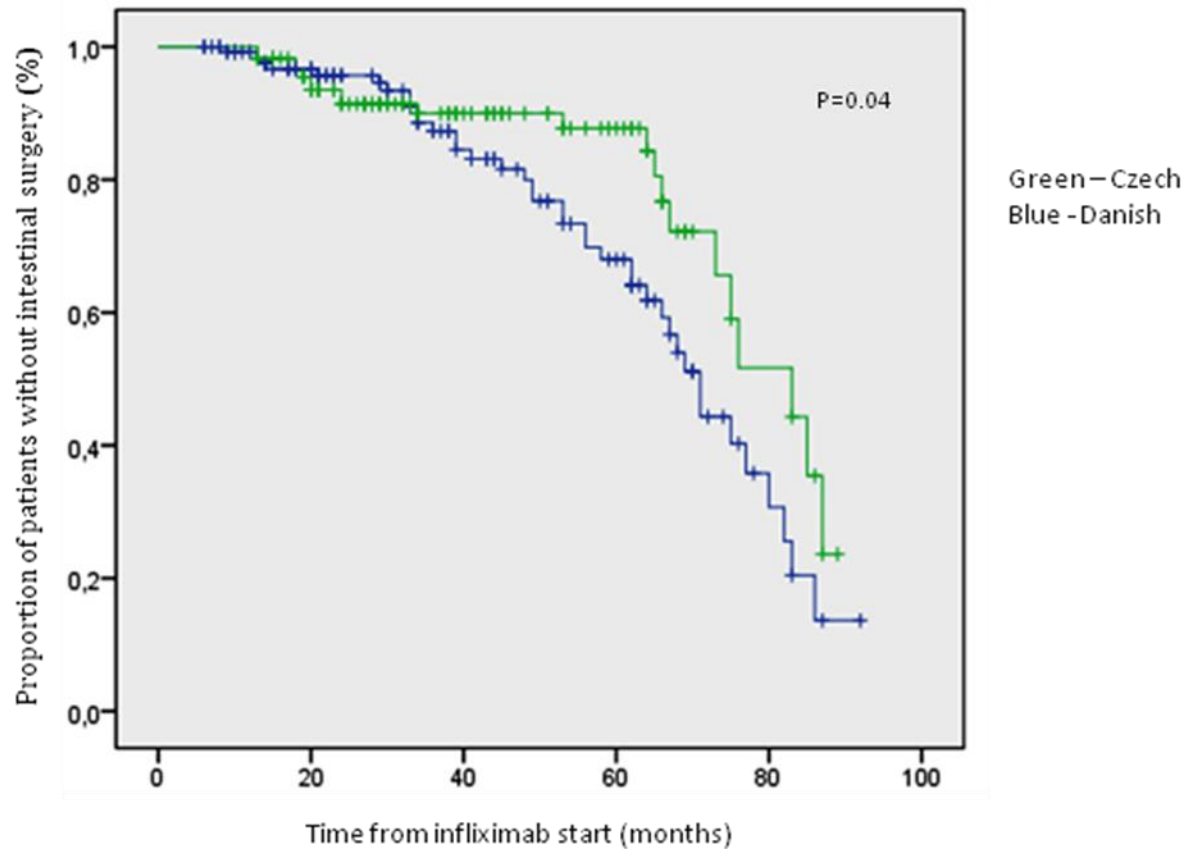
6.3   Paper III

Five hundred thirty-seven patients with CD from DCCD were identified to be treated at Herlev University Hospital. In 7 patients medical records were not available and 185 were either never treated with 5-ASA or the information about previous 5-ASA treatment was missing due to incomplete medical records. Of the rest 345 patients treated with 5-ASA, 165 fulfilled the inclusion criterion and were assessed according to phenotype model of 5-ASA dependency. The median (range) follow-up since 5-ASA monotherapy start was 137 months (6-670). Proportion of included patients per decade of diagnosis is shown in Table 10 and the clinical and demographic characteristics at start of 5-ASA monotherapy are outlined in Table 11.

Table 10. Proportion of CD patients on 5-ASA monotherapy with respect to decade of diagnosis

Decade of diagnosis	n=165
1953 – 1969	4 (2%)
1970 – 1979	12 (7%)
1980 – 1989	34 (21%)
1990 – 1999	69 (42%)
2000 – 2007	46 (28%)

Table 11. Demographic and clinical characteristics of CD patients at start of 5-ASA monotherapy

	n=165
Male (%)	71 (43)
Age (yr): median (range)	33 (13-83)
Disease duration (yr): median (range)	0 (0-49)
Disease localization (%)	
Terminal ileum	45 (27)
Colon	79 (48)
Ileo-colon	29 (18)
Upper disease +/- ileum and/or colon	8 (5)
Unknown	4 (2)
Disease behaviour (%)	
Inflammatory	146 (88)
Stricturing	6 (4)
Penetrating	12 (7)
Unknown	1 (1)
Perianal disease at any time prior to 5-ASA monotherapy (%)	21 (13)
Intestinal surgery prior to 5-ASA course (%)	30 (18)

6.3.1 5-ASA treatment outcome

The immediate and long-term outcome of the 1st course of 5-ASA monotherapy is presented in Figure 8. The median duration of 5-ASA course was 34 months (range: 1-304) in patients with prolonged response, 63 (6-336) in 5-ASA dependent patients and 2 (0-10) in non-responders. The daily dose of 5-ASA ranged from 1 to 4.8g.

Five (3%) patients were not assessed in long-term outcome due to a short treatment course.

Figure 8. The outcome of the 1st treatment course with 5-ASA in patients with CD
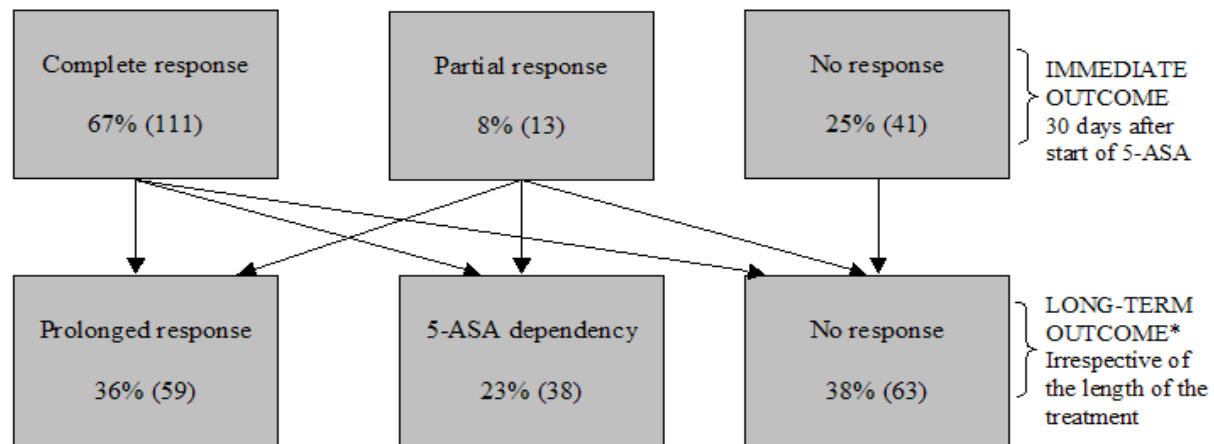
Kaplan-Meier analysis illustrates the cumulative probability of the first intestinal surgery since the diagnosis with respect to long-term outcome (Figure 9). 5-ASA dependent patients had significantly lower surgical risk than non-responders (p=0.007); no significant difference was observed between prolonged responders and non-responders (p=0.1).

Figure 9. Proportion of CD patients without intestinal surgery since diagnosis with respect to long-term outcome of 5-ASA monotherapy
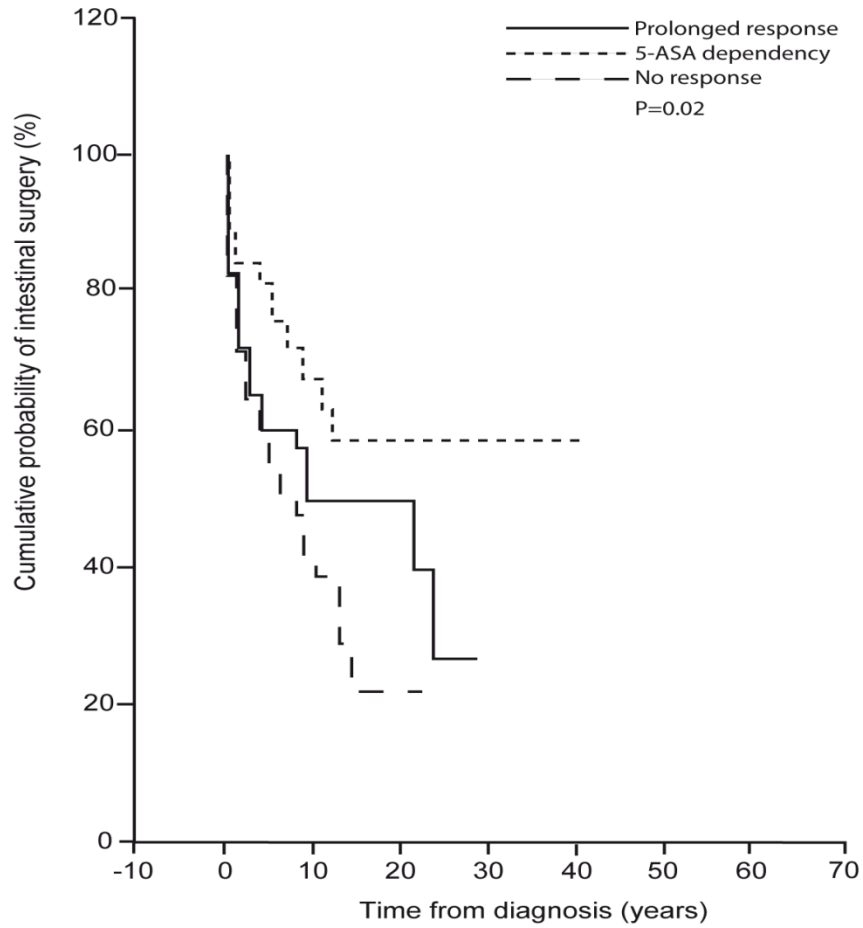
Number of patients at risk

Prolonged response	59	19	6	0	0
5-ASA dependency	38	15	6	1	0
No response	63	14	2	0	0

6.3.2 Predictors of 5-ASA response

Logistic regression analyses identified female gender as predictor of favourable long-term outcome - prolonged response or 5-ASA dependency (OR 2.89, 95%CI: 1.08-7.75).

Focusing on 5-ASA dependent response, patients with longer disease duration were more likely to become 5-ASA dependent (OR 4.06, 95%CI: 1.09-15.1) (Table 12).

Table 12. Clinical predictors of long-term outcome of 5-ASA monotherapy in CD patients with immediate complete or partial response (n=119)

	Prolonged response & 5-ASA dependency	5-ASA dependency
Predictor factor	OR (95%CI)	OR (95%CI)
Male gender	0.33 (0.12 –0.92)*	1.19 (0.42-7. 3.34)
Age at IFX start >40 yr vs. ≤40 yr	0.58 (0.19 - 2.1.74)	0.55 (0.18-1.65)
Disease duration >3 yr vs. ≤3 yr	1.27 (0.32 – 5.10)	4.06 (1.09-15.09)*
Disease localization - Colonic/ileocolonic vs. Ileal - Upper disease	1.78 (0.57-5.55) 0.29 (0.03-3.20)	2.87 (0.82-10.0) 0.78 (0.02-33.39)
Behaviour - Non-inflammatory vs. Inflammatory	0.47 (0.14-1.65)	0.53 (0.05-5.93)
Prior intestinal surgery	0.58 (0.13-2.62)	0.54 (0.11-2.65)

OR, odds ratio; CI, confidence interval; *statistically significant results

7   DISCUSSION

IBD is a complex immunopathological disorder of unknown aetiology involving several inflammatory mechanisms of different importance in inflammatory process. Based on various clinical presentations the disease seems to be rather a group of heterogeneous entities having probably more or less different pathophysiological background. This heterogeneity may be seen also in wide inter-individual variability in drug response resulting in several drug response phenotypes. Whereas some patients respond completely, others experience only improvement of the symptoms and a proportion of individuals have no response at all. In the long-term perspective, loss of initially good response occurs in some patients while the others develop dependency to the drug and can´t be weaned off the drug due to a prompt relapse of the disease after its cessation or dose decrease. The pathophysiological mechanisms responsible for these response phenotypes are being studied and so far are only partially understood.

Ten to 35% of IBD patients do not respond to anti-TNFα therapy being primary non-responders (100;155;178-180). Although TNFα seems to play a central role in inflammatory process in majority of individuals, several different, probably TNFα independent inflammatory pathways involved in inflammation of IBD have been identified (116). It is likely that these alternative inflammatory mechanisms dominate in patients with primary non-response and TNFα plays only minor or no role at all.

Eleven to 50% of patients lose their response during the treatment course being secondary anti-TNFα failures (181;182). Hypothetically, two different situations may be responsible for loss of response to anti-TNFα therapy: 1) pharmacokinetic mechanisms leading to low serum drug levels; b) loss of dominancy of pharmacodynamic effect of these preparations in inflammatory process.
Low serum trough levels of both IFX and adalimumab have been associated with worse treatment outcome in term of clinical response, endoscopic healing, length of sustained response and surgery rate as compared to therapeutic drug levels (183-186). Several mechanisms resulting in an increased clearance of the drug may be involved:

Anti-TNFs are protein molecules with immunogenic potential leading to anti-drug antibodies formation. IFX, due to its chimeric molecular structure is more immunogenic than fully human adalimumab with a reported frequency of anti-drug antibodies of 0.9-61% and 0.04-17% for IFX and adalimumab, respectively (100;184-191). Formation of anti-drug antibodies has been thought to be an important mechanism responsible for increased clearance of the drug and subsequent its low serum concentrations. Nevertheless, a substantial proportion of patients have low serum drug levels with negative antibodies suggesting also other mechanism to be involved. Higher consumption of IFX due to high TNFα "load" has been suggested as another factor contributing to low serum IFX levels (102). In a study of rheumatoid arthritis patients published by Bendtzen et al., individuals with higher pre-treatment disease activity had lower initial IFX levels than "less sick" patients, with this difference disappearing later in the treatment course (102). The authors hypothesized that higher TNFα burden in sicker patients might have lead to higher IFX clearance and subsequent lower drug levels (102).

Finally, anti-TNFα antibodies are proteins which to some extent share metabolic pathways with serum immunoglobulins and other proteins. Active inflammation is associated with enhanced protein catabolism and increased production of pro-inflammatory molecules instead; furthermore there is an increased loss of proteins via inflamed mucosa. A good correlation between IFX levels and serum albumin has been shown (192;193). Serum albumin and IgG immunoglobulins share common receptor (neonatal Fc receptor – FcRn) which is involved in their homeostasis and protects albumin and IgGs from the catabolism (192). Hence, serum albumin level has been proposed as an indicator of a degree of catabolism of immunoglobulines including IFX antibodies (192;193).

The impact of the drug levels on anti-TNFα efficacy may be well illustrated by possibility to regain the response in some anti-TNFs failures using treatment intensification. The principle of this approach is via increasing the dose and/or shortening the interval between drug applications to increase the serum level of the drug. So far, intensification seems to work best in individuals with a low or no levels of the drug (194-196).

Nevertheless, it has to be emphasized that although patients with therapeutic drug levels seem to have better overall treatment outcome, the pharmacokinetic parameters of anti-TNFs do not have to relate necessarily to clinical response. Fourteen to 27% of patients on IFX have good long-term response despite subtherapeutic or undetectable IFX levels (185;186). On the other hand a

proportion of patients lose their response despite high serum levels of the drug (194). This corroborates the complexity of mechanism of action of these preparations.

As mentioned above several other TNFα independent inflammatory pathways have been identified to mediate inflammation in IBD as well (116). One can hypothesize that a shift of a dominant mechanism of inflammation from preferentially TNFα dependent to mainly TNFα independent may be responsible for loss of pharmacodynamic effect of anti-TNFs and subsequent loss of response (116). This situation might play the central role in individuals who become anti-TNFα failures despite high levels of the drug in serum.

Moreover, anti-TNFα therapy *per se* might have a potential to induce or up-regulate other pro-inflammatory pathways which may also contribute to secondary anti-TNFα failure (116). Another consequence of activation of non-TNFα inflammatory mechanisms is development of so called "paradoxical inflammatory events" in individuals with immune mediated inflammatory disorders such as IBD or rheumatologic diseases. There is an increasing evidence of de novo occurrence of rheumatologic problems, psoriasis or psoriasis-like skin disorders in patients with IBD while on anti-TNFs (197-199). Similarly, new onset of IBD has been reported in rheumatologic patients treated with these monoclonal antibodies (200).

Recently a new response phenotype to IFX therapy, IFX dependency, has been described (136;139). IFX dependent patients are very good responders who, however relaps quickly once the drug is stopped or dose decreased. This is in contrast to patients with prolonged response who also respond well to the drug, but maintain the remission even after drug cessation. So far, no pathophysiological concept for IFX dependency has been proposed. Hypothetically, this difference in their ability to sustain the remission upon drug stop might be given by the difference in durability of the pharmacodynamic effect. While it might lead to a long-term down-regulation of the inflammatory process in prolonged responders it results only in a time limited effect in dependent individuals. Nevertheless, the inflammation seems to be mediated all over by the same inflammatory mechanisms since dependent patients respond repetitively once the drug is re-administered. There is far more unknown than known and future basic studies should address these questions.

The results of our study revealed that 66% and 29% of paediatric and adult CD patients respectively became IFX dependent. To date, two studies of children cohorts have been published evaluating the frequency of IFX dependency and reported that 42% and 56% of children developed IFX dependency (136;139). Our finding of 66% of dependent children is in agreement with the high frequency of this response phenotype observed in the previous studies. However, the results of adult population indicate a lower frequency of IFX dependency compared with children cohorts. The potential explanation could be that paediatric IBD seems to have different phenotype than adult onset-disease with more extensive involvement and aggressive disease course (201;202). We can only speculate that the differences between paediatric and adult cohorts might be due to the different natural disease courses in these two populations.

Significantly more Danish than Czech patients developed IFX dependency. The significant difference in treatment regimes between the two countries seems to be the probable cause of this observation. Nevertheless, the treatment policy which was discussed prior to study start assumed to be similar in both cohorts. Furthermore, if the potential non-availability of IFX with subsequent more induction regimes only was the reason of low frequency of IFX dependency in Czech cohort, one would expect that patients with a good initial response and early relapse after IFX stop but without IFX re-introduction would have been considered non-responders as per definition. However, no significant difference in non-responders was observed, in contrast Czech patients maintained significantly more prolonged response than Danish ones (data not shown). Furthermore, Czech patients had lower cumulative probability of surgery after IFX start than Danish patients. We thus hypothesize that other factors such as potential referral centre bias regarding disease severity at IFX start could contribute to this observation.

In children cohort, inflammatory disease behavior was associated with significantly better long-term outcome compared to complicated disease. This is in agreement with the finding that surgery naïve patients prior to IFX start, assumed to have mainly non-stricturing/non-penetrating disease, were more likely to become prolonged responders or IFX dependent.

Children with perianal fistula at IFX start had higher probability to become IFX dependent. Perianal disease has varied complexity and severity with an impact on therapeutic result and deep and permanent healing of all tracks is an important assumption of sustained response (203). The superficial healing or premature closure with remaining deep tracks leading to early and

recurrent relapses might partly explain our finding. Contrary to our result, the national paediatric study from Netherlands reported higher rate of IFX dependency in children without fistulas than in those with fistulizing disease (136). No relevant clinical predictor was identified in adult cohort.

No genetic predictor was identified in paediatric cohort, while two variants were found in our study of adult population. In Danish patients, the presence of G allele of *LTA* c.207 A>G variant was associated with better long-term IFX outcome. Taken in account the results by Taylor et al. (204) who reported a certain haplotype of lymphotoxin alpha (LTA) to be responsible for a decreased response to IFX in CD, this could suggest a possible role of *LTA* variants in prediction of IFX outcome. However, this finding could not be replicated in the Czech cohort. In Czech patients, we revealed an association of T allele of *CASP9* c.93 C>T variant with IFX dependent phenotype. Similarly, Hlavaty *et al*. (205) have previously reported an association of TT genotype of *CASP9* c.93 C>T variant with positive short-term response to IFX. No such association was found in Danish patients, despite the same frequency of this variant in both background populations.

The *TNF*−308 A>G variant has been shown to influence the production of TNF-α (206) and to be associated with disease activity of CD (206;207). Nevertheless, no association of variants within genes encoding TNF-α or its receptor with IFX outcome has been proven in previously published studies (208;209), nor has our study proven it. *FASLG c.-844 C>T* variant of *FasL/Fas* system involved in apoptosis has been shown to have an influence on IFX outcome (205), however our study failed to reveal any association of this variant with IFX response.

Patients with prolonged response as well as IFX dependent individuals were shown to have lower cumulative probability of surgery compared to non-responders. Of note is that IFX dependent children had even significantly lower risk of surgery than those with prolonged response. The question whether biological therapy is able to modify the disease course and decrease the need for surgical interventions is still unclear. So far, there is only a little evidence that IFX might reduce the surgery rate (210-213). Our findings show that the need for surgery in both prolonged responders and IFX dependent patients may be at least postponed if not avoided.

The major limitation of our IFX dependency studies is the retrospective character bearing the risk of data validity and misinterpretation. The study design also precluded assessment of other

factors of clinical interest such as smoking or disease activity. On the other hand retrospective studies provide reflection of the everyday clinical practice and thus have their role in evidence. Furthermore, the treatment regimes used in the two countries were different. This happened despite the fact that the treatment strategy was discussed prior to study start and seemed to be similar. Nevertheless, when finally analyzing the data we found that that there was a difference in treatment approach between the countries. Regarding genetic analyses, the study populations were quite small (mainly in paediatric cohort) which could be a reason that possible associations were not detected. Moreover, no adjustment for other confounders (such as smoking, disease related characteristics) was performed due to relatively small numbers of subjects. Therefore our results should be interpreted with caution and larger independent cohort is needed to confirm or disprove these findings.

Dependent response phenotype has been also described for another drug used in the treatment of IBD, 5-ASA. This phenotype has been defined and described for the first time in our study. Efficacy of 5-ASA in treatment of CD is considerably lower compared to corticosteroids or IFX with the number needed to treat of 10 and 13 for induction and maintenance of remission (214). Despite this fact the results of our study demonstrated that there is a group of CD patients with a nice response to 5-ASA preparations. Of 165 patients who started on 5-ASA monotherapy 59% obtained long-term benefit (prolonged response or 5-ASA dependent) with 23% being 5-ASA dependent. 5-ASA dependent patients contrary to prolonged responders represented a specific group having a relapse of the disease repetitively responding to 5-ASA and thus confirming efficacy of the drug. The results of our study may seem too favourable; however, it has to be emphasized that a selected group was analysed (31% of all CD patients treated at Herlev hospital). Thus, the total number of CD patients with a long-term benefit of 5-ASA was not high, as one would expect. Nevertheless, 5-ASA preparations are one of the safest therapeutics used in IBD and if 5-ASA was not used in CD any longer these patients would have to be treated with other, more potent but also more toxic anti-inflammatory agents.

The role of 5-ASA in CD was also demonstrated in a recent retrospective study from Germany (215). In that study 103 newly diagnosed CD patients from 12 gastroenterology centres, followed-up for at least 12 months were included. The authors observed that 15% of all patients had a mild disease course treated only with mesalazine therapy and of them almost 50% were

maintained only on 5-ASA up to 48 months without need of "stronger" anti-inflammatory agents. Older age at diagnosis, lower inflammatory activity expressed by lower C-reactive protein and lack of severe endoscopic findings were associated with this mild disease course (215).

In our study, female gender was identified as a predictor of better long-term response to 5-ASA. This might be explained by the findings of previous study reporting lower adherence with maintenance 5-ASA medication in males (216). Focusing on 5-ASA dependent phenotype, patients with longer disease duration were more likely to become dependent. Theoretically, this finding may be rather a reflection of a very mild disease phenotype. This speculation might be also supported by our results of significantly lower cumulative probability of surgery in 5-ASA dependent patients compared to non-responders, which was not observed in case of prolonged responders.

Our study of 5-ASA dependency has several limitations. First limitation is the retrospective character carrying the risk of misinterpretation of the treatment response which was based on medical records only. Secondly, the adherence to 5-ASA could not be assessed and was assumed only by the record that the patient was taking the drug. Furthermore, other factors such as smoking which might have had an impact on disease course were not assessed due to study design. Finally, different 5-ASA preparations and doses were used with a possible impact on the results, although one would expect worse treatment outcome in case of low doses or unsuitable drug forms.

Contrary to CD, 5-ASA works well in mild to moderate UC (164;165;172-174). The reason for this difference is unknown and is likely to be in the different pathophysiologic origin of the diseases. 5-ASA seems to exert its anti-inflammatory efficacy by local effect on colonic mucosa irrespective of systemic serum concentrations (217;218). There is a relationship between mucosal 5-ASA levels and endoscopic activity with higher 5-ASA tissue concentrations being associated with better mucosal healing (217;218). Theoretically, the relative infectivity of 5-ASA in CD compared to UC might be based on the fact that while UC affects mucosa only, CD is a transmural disease involving also deeper layers of the bowel. Nevertheless, as shown by our and other studies there are some patients who benefit from 5-ASA having probably very mild disease inflammatory activity.

Similarly to anti-TNFs and 5-ASA, response to corticosteroids varies among the individuals. Over 80% of IBD patients respond primarily to corticosteroid therapy, either completely or partially (78). Nevertheless, of primary responders a proportion of patients lose their response, while 22% to 36% of adult individuals with IBD become steroid dependent (78;137).

So far, several mechanisms of this variable sensitivity to corticosteroids have been proposed, nevertheless the exact mechanism is still not known. Based on complexity of corticosteroid action the responsiveness to the drug may be impacted at several levels of corticosteroid signalling.

Intracellular receptor for corticosteroids has been proposed as the first place responsible for steroid resistance. To date, several polymorphisms in gene encoding the receptor have been described with only a few seem to be relevant. Three polymorphisms - T*thIII*I, ER22/23EK and GR-9β - have been identified as potentially associated with reduced sensitivity to endogenous and exogenous glucocorticoids (81). Nevertheless, their role with regard to corticosteroid response in IBD or other diseases has not been largely studied yet and a study on paediatric IBD patients failed to show any association between ER22/23EK polymorphism and response to corticosteroids (219). Inversely, a variant of receptor gene B*cl*I has been linked to an increased sensitivity to corticosteroids (81). In an Italian study of IBD children (219), a significant association between B*cl*I polymorphism and corticosteroid response was found with a higher frequency of mutated genotype in children with corticosteroid dependency than in steroid responsive non-dependent individuals (OR 5.94, 95%CI: 1.23-28.6). Glucocorticoid receptor is a heterocomplex existing in a complex with several protein components which keep receptor in optimal conformation for ligand binging (81). Impairment in these components has been found in individuals with steroid resistant asthma, sclerosis multiplex and idiopathic nephritic syndrome (81). Their association with corticoresistant IBD has not been studied yet (81).

Translocation of corticosteroid-receptor complex to nucleus is mediated via specific nuclear transport factors – importins (81). Their alteration thus seem to be other *locus minoris* for steroids resistance although their role in corticosteroid response in IBD or other diseases has been studied only a little or not at all and needs further investigation (81).

There is evidence that several cytokines influence effect of corticosteroids trough interference with the corticosteroid signalling pathway. For example, TNFα and IL-2 have negative impact on steroid action while IL-10 acts inversely (81). Furthermore, higher levels of TNFα and other pro-

inflammatory cytokines have been described in mucosa of UC patients resistant to corticosteroids (118). Interestingly the A allele of G-308A variant of TNF gene which has been linked to an increased production of TNFα has been reported to be associated with steroid dependency in a population of CD patients (220). Furthermore, in another study carries of A allele of G-308A variant have been found to be more likely resistant to corticosteroids than individuals with GG genotype (OR 0.29, 95%CI: 0.08-0.98) (221). No association with steroid dependency was revealed in that study.

Finally, the role of P-glycoprotein and the polymorphisms of its encoding gene MDR1 (multi drug resistance) have been studied with regard to response to corticosteroids (81;82;222). P-glycoprotein serves as a cell pump which transports xenobiotics and different drugs out of the cells and thus may decrease their efficacy (81;82;222). While some studies have shown an association between MDR1 variants and corticosteroid response, other failed to confirm these findings (221;223-225).

Drug dependency, connected with corticosteroids mainly, used to be considered negatively. However, it can be also a positive response outcome which may offer the patient some benefits. To decide its implication in disease prognosis, the safety and the efficacy of the drug have to be evaluated.

Corticosteroids are strong anti-inflammatory agents with a high response rate and prompt onset of clinical effect (214). However, their use is associated with a wide range of serious adverse events including growth impairment in children (79). Moreover, in long-term perspectives corticosteroids seem to carry relatively high risk of surgery as 27%-38% of CD patients have been reported to undergo surgery within one year after drug cessation (137;143). More favourable results, however, may be found in recent paediatric population-based study from Denmark (138). The study showed that despite the high frequency of steroid dependency in their cohort (37% in CD and 49% in UC), the cumulative risk of surgery within the first year after steroids start was relatively low for both CD and UC (11.5% and 7.8% respectively) with no difference between steroid dependent and non-dependent individuals. This finding is probably related to high rate of early introduced immunomudulators in this cohort (80%). Similar observations can be found also in other recent paediatric studies (141;226).

IFX shows similar short term efficacy as corticosteroids (214) but contrary to steroids IFX seems to have also long-term benefit in terms of disease activity control, reduced number of hospitalizations and perhaps also decreased need for surgery (163;211-213;227). Thus, from this point of view and also based on results of our studies, IFX dependency seems to be a disease phenotype with a good prognosis.

Despite the evident clinical benefit of IFX, the treatment, mainly in long-term term use is associated with certain drawbacks. As already mentioned, the potential complications might be very serious and life-threatening (157;158;160), further there is an increasing evidence of probably immunopathological complications induced by biological preparations such skin and joint problems (199;228;229). Moreover, there is no data about the consequences of the long-standing use of IFX yet. No less, the therapy is very expensive and represents a big economic burden (162;163). Therefore, identification of IFX dependent individuals may be very useful in clinical management. It may prevent "overtreatment" of non-dependent individuals, and thus limit potential toxicity in these patients. Furthermore, overall cost-effectiveness of IFX treatment may be improved.

5-ASA in treatment of CD compared to UC has only limited efficacy. But as obvious from our and also other studies, there is a population of CD patients with a very mild disease course which can be managed just by 5-ASA preparations. 5-ASA dependent individuals seem to represent a specific group among these patients maintaining disease remission rather by 5-ASA than natural disease evolution which probably plays more or less important role in patients with prolonged response. Since 5-ASA preparations have a very low toxicity, 5-ASA dependency might be seen as a favourable condition with a good prognosis. Self-management of the disease has been proposed to optimize patient adherence to therapy and reduce the healthcare costs (230;231). Recently, a very interesting study has been published dealing with web-based self-treatment of UC patients with 5-ASA preparations (14). The study has shown the benefit of this approach compared to "classic" one in terms of reduction of relaps duration, cost-effectiveness, improvement of treatment adherence and quality of life. 5-ASA preparations with their good safety profile would represent an ideal drug for disease self-management which might be a suitable therapeutic approach for 5-ASA dependent individuals.

Drug dependency is more than just a simple response pattern; it is a specific disease phenotype with a certain prognostic value which depends on particular drug. Hence, it can be used as a helpful prognostic marker in everyday clinical management. Early identification of dependent individuals might prevent negative consequences of steroid therapy; avoid "unnecessary" use of infliximab in non-dependent individuals, lead to better treatment cost-effectiveness and limit drug toxicity by use of 5-ASA in those profiting from these preparations.

8   CONCLUSIONS

In these studies, IFX dependency in children with CD was assessed and for the first time IFX dependency in adult cohort with CD and 5-ASA dependency in CD patients were studied.

The frequency of IFX dependency in children was found to be similarly high as in the previous paediatric studies, whereas a lower rate was observed in adult cohort. In children, perianal disease and no bowel surgery prior to IFX start were predicative of IFX dependency, while 2 genetic variants *LTA* c.207 A>G and *CASP9* c.93 C>T were associated with IFX outcome in adult population. Future studies, however, have to assess the relevance of these findings.

Probably, the most relevant for the clinicians and CD patients was a finding of a significant decrease in surgical rate after IFX initiation which was observed in both prolonged responders and IFX dependent patients, in paediatric as well as in adult cohort.

5-ASA therapy was shown to be efficacious in a subgroup of patients with CD. Fifty-nine percent of included patients had long-term benefit of 5-ASA and 23% of them were 5-ASA dependent. Women were more likely to have a good response to 5-ASA than men. 5-ASA dependent patients were characterized by a very mild disease course reflected by the lowest surgical rate.

Drug dependency is a specific disease phenotype which determines patient´s prognosis. Hence, it may be used as a prognostic marker when deciding for treatment management. Whereas corticosteroid dependency itself has a negative impact on disease course, IFX and 5-ASA dependency seem to have a good prognosis. Prediction of the clinically relevant dependent patient still needs to be explored. The developing web-based treatment programmes for patient self-management based on individual disease course might be an option in the future.

9   PERSPECTIVES

The use of "dependency definitions" in clinical practice is problematic. One reason is their heterogeneity. The other reason are the over time changing treatment recommendations. Currently, immunomodulators are introduced earlier to avoid corticosteroid dependency and IFX is indicated as long-term maintenance therapy in all patients responding to it (79;88). Thus, the frequency of drug dependency may be biased. Further, the doctor preference and economical possibilities have to be also considered. Hence, it is important that the definitions also reflect these changes in clinical practice and are adjusted according to the actual requirements.

Early identification of mainly IFX dependent individuals may seem to be challenging as IFX is recommended as long-term maintenance treatment in all individuals responding to induction therapy. The developing web based treatment systems which offer patients self management according to their individual needs provide a good picture of the patient´s disease course and may thus in the future help to identify dependent individuals (15).

Future studies should assess the frequency of IFX and 5-ASA dependency also in UC patients. Furthermore, other drugs used in IBD such as adalimumab and azathioprine should be studied for drug dependency and their prognostic value assessed.

REFERENCE LIST

1.   Podolsky DK. Inflammatory bowel disease. N Engl J Med. 2002;347:417-429.

2.   Langholz E, Munkholm P, Krasilnikoff PA, et al. Inflammatory bowel diseases with onset in childhood. Clinical features, morbidity, and mortality in a regional cohort. Scand J Gastroenterol. 1997;32:139-147.

3.   Vind I, Riis L, Jess T, et al. Increasing incidences of inflammatory bowel disease and decreasing surgery rates in Copenhagen City and County, 2003-2005: a population-based study from the Danish Crohn colitis database. Am J Gastroenterol. 2006;101:1274-1282.

4.   Cosnes J, Gower-Rousseau C, Seksik P, et al. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology. 2011;140:1785-1794.

5.   Moum B, Ekbom A, Vatn MH, et al. Change in the extent of colonoscopic and histological involvement in ulcerative colitis over time. Am J Gastroenterol. 1999;94:1564-1569.

6.   Munkholm P, Langholz E, Davidsen M, et al. Disease activity courses in a regional cohort of Crohn's disease patients. Scand J Gastroenterol. 1995;30:699-706.

7.   Langholz E, Munkholm P, Davidsen M, et al. Changes in extent of ulcerative colitis: a study on the course and prognostic factors. Scand J Gastroenterol. 1996;31:260-266.

8.   Cosnes J, Cattan S, Blain A, et al. Long-term evolution of disease behavior of Crohn's disease. Inflamm Bowel Dis. 2002;8:244-250.

9.   Sands BE, Arsenault JE, Rosen MJ, et al. Risk of early surgery for Crohn's disease: implications for early treatment strategies. Am J Gastroenterol. 2003;98:2712-2718.

10.   Solberg IC, Vatn MH, Hoie O, et al. Clinical course in Crohn's disease: results of a Norwegian population-based ten-year follow-up study. Clin Gastroenterol Hepatol. 2007;5:1430-1438.

11.   Pierik M, Rutgeerts P, Vlietinck R, et al. Pharmacogenetics in inflammatory bowel disease. World J Gastroenterol. 2006;12:3657-3667.

12.   Dubinsky M. Can serologic markers help determine prognosis and guide therapy? Dig Dis. 2010;28:424-428.

13.   Louis E, Belaiche J, Reenaers C. Do clinical factors help to predict disease course in inflammatory bowel disease? World J Gastroenterol. 2010;16:2600-2603.

14.   Elkjaer M, Shuhaibar M, Burisch J, et al. E-health empowers patients with ulcerative colitis: a randomised controlled trial of the web-guided 'Constant-care' approach. Gut. 2010;59:1652-1661.

15.   Pedersen N, Elkjaer M, Duricova D, et al. Ehealth: Optimization of infliximab treatment and disease course via self-initiated web-based solution in Crohn´s disease. Journal of Crohn´s and Colitis. 2011;S84.

16.   Armitage EL, Aldhous MC, Anderson N, et al. Incidence of juvenile-onset Crohn's disease in Scotland: association with northern latitude and affluence. Gastroenterology. 2004;127:1051-1057.

17.   Jakobsen C, Paerregaard A, Munkholm P, et al. Pediatric inflammatory bowel disease: Increasing incidence, decreasing surgery rate, and compromised nutritional status: A prospective population-based cohort study 2007-2009. Inflamm Bowel Dis. 2011.

18.   Loftus EV, Jr., Schoenfeld P, Sandborn WJ. The epidemiology and natural history of Crohn's disease in population-based patient cohorts from North America: a systematic review. Aliment Pharmacol Ther. 2002;16:51-60.

19.   Turunen P, Kolho KL, Auvinen A, et al. Incidence of inflammatory bowel disease in Finnish children, 1987-2003. Inflamm Bowel Dis. 2006;12:677-683.

20.   Lakatos PL. Recent trends in the epidemiology of inflammatory bowel diseases: up or down? World J Gastroenterol. 2006;12:6102-6108.

21.   Strober W, Fuss IJ. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. Gastroenterology. 2011;140:1756-1767.

22.   Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. J Clin Invest. 2007;117:514-521.

23.   Sartor RB. Microbial influences in inflammatory bowel diseases. Gastroenterology. 2008;134:577-594.

24.   Koloski NA, Bret L, Radford-Smith G. Hygiene hypothesis in inflammatory bowel disease: a critical review of the literature. World J Gastroenterol. 2008;14:165-173.

25.   Brant SR. Update on the heritability of inflammatory bowel disease: the importance of twin studies. Inflamm Bowel Dis. 2011;17:1-5.

26.   Jess T, Riis L, Jespersgaard C, et al. Disease concordance, zygosity, and NOD2/CARD15 status: follow-up of a population-based cohort of Danish twins with inflammatory bowel disease. Am J Gastroenterol. 2005;100:2486-2492.

27.   Orholm M, Munkholm P, Langholz E, et al. Familial occurrence of inflammatory bowel disease. N Engl J Med. 1991;324:84-88.

28.   Cho JH, Brant SR. Recent insights into the genetics of inflammatory bowel disease. Gastroenterology. 2011;140:1704-1712.

29.   Lees CW, Barrett JC, Parkes M, et al. New IBD genetics: common pathways with other diseases. Gut. 2011;60:1739-1753.

30.   Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature. 2001;411:599-603.

31.   Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature. 2001;411:603-606.

32.   Economou M, Trikalinos TA, Loizou KT, et al. Differential effects of NOD2 variants on Crohn's disease risk and phenotype in diverse populations: a metaanalysis. Am J Gastroenterol. 2004;99:2393-2404.

33.   Ahmad T, Armuzzi A, Bunce M, et al. The molecular classification of the clinical manifestations of Crohn's disease. Gastroenterology. 2002;122:854-866.

34.   Vind I, Vieira A, Hougs L, et al. NOD2/CARD15 gene polymorphisms in Crohn's disease: a genotype-phenotype analysis in Danish and Portuguese patients and controls. Digestion. 2005;72:156-163.

35.   Fritz T, Niederreiter L, Adolph T, et al. Crohn's disease: NOD2, autophagy and ER stress converge. Gut. 2011;60:1580-1588.

36.   Kanneganti TD, Lamkanfi M, Nunez G. Intracellular NOD-like receptors in host defense and disease. Immunity. 2007;27:549-559.

37.   Meylan E, Tschopp J, Karin M. Intracellular pattern recognition receptors in the host response. Nature. 2006;442:39-44.

38.   Abraham C, Medzhitov R. Interactions between the host innate immune system and microbes in inflammatory bowel disease. Gastroenterology. 2011;140:1729-1737.

39.   Levine B, Deretic V. Unveiling the roles of autophagy in innate and adaptive immunity. Nat Rev Immunol. 2007;7:767-777.

40.   Cadwell K, Liu JY, Brown SL, et al. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. Nature. 2008;456:259-263.

41.   Barrett JC, Hansoul S, Nicolae DL, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet. 2008;40:955-962.

42.   Parkes M, Barrett JC, Prescott NJ, et al. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. Nat Genet. 2007;39:830-832.

43.   Cooney R, Baker J, Brain O, et al. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. Nat Med. 2010;16:90-97.

44.   Travassos LH, Carneiro LA, Ramjeet M, et al. Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. Nat Immunol. 2010;11:55-62.

45.   Eckburg PB, Relman DA. The role of microbes in Crohn's disease. Clin Infect Dis. 2007;44:256-262.

46.   Sellon RK, Tonkonogy S, Schultz M, et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun. 1998;66:5224-5231.

47.   Veltkamp C, Tonkonogy SL, De Jong YP, et al. Continuous stimulation by normal luminal bacteria is essential for the development and perpetuation of colitis in Tg(epsilon26) mice. Gastroenterology. 2001;120:900-913.

48.   Chassaing B, Darfeuille-Michaud A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. Gastroenterology. 2011;140:1720-1728.

49.   Darfeuille-Michaud A, Neut C, Barnich N, et al. Presence of adherent Escherichia coli strains in ileal mucosa of patients with Crohn's disease. Gastroenterology. 1998;115:1405-1413.

50.   Rutgeerts P, Goboes K, Peeters M, et al. Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. Lancet. 1991;338:771-774.

51.   D'Haens GR, Geboes K, Peeters M, et al. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. Gastroenterology. 1998;114:262-267.

52.   Duricova D, Bortlik M, Lukas M. Rifaximin v terapii Crohnovy nemoci. Výsledky studie GRACE 02. Ces a Slov Gastroent a Hepatol. 2010;64:31-36.

53.   Gionchetti P, Rizzello F, Helwig U, et al. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. Gastroenterology. 2003;124:1202-1209.

54.   Rutgeerts P, Hiele M, Geboes K, et al. Controlled trial of metronidazole treatment for prevention of Crohn's recurrence after ileal resection. Gastroenterology. 1995;108:1617-1621.

55.   Dubinsky M. What is the role of serological markers in IBD? Pediatric and adult data. Dig Dis. 2009;27:259-268.

56.   Manichanh C, Rigottier-Gois L, Bonnaud E, et al. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut. 2006;55:205-211.

57.   Swidsinski A, Ladhoff A, Pernthaler A, et al. Mucosal flora in inflammatory bowel disease. Gastroenterology. 2002;122:44-54.

58.   Strober W, Murray PJ, Kitani A, et al. Signalling pathways and molecular interactions of NOD1 and NOD2. Nat Rev Immunol. 2006;6:9-20.

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

60.   Munkholm P, Langholz E, Hollander D, et al. Intestinal permeability in patients with Crohn's disease and ulcerative colitis and their first degree relatives. Gut. 1994;35:68-72.

61.   Braun A, Treede I, Gotthardt D, et al. Alterations of phospholipid concentration and species composition of the intestinal mucus barrier in ulcerative colitis: a clue to pathogenesis. Inflamm Bowel Dis. 2009;15:1705-1720.

62.   Nuding S, Fellermann K, Wehkamp J, et al. Reduced mucosal antimicrobial activity in Crohn's disease of the colon. Gut. 2007;56:1240-1247.

63.   Wehkamp J, Harder J, Weichenthal M, et al. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. Gut. 2004;53:1658-1664.

64.   Wehkamp J, Salzman NH, Porter E, et al. Reduced Paneth cell alpha-defensins in ileal Crohn's disease. Proc Natl Acad Sci U S A. 2005;102:18129-18134.

65.   Elson CO, Cong Y, McCracken VJ, et al. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. Immunol Rev. 2005;206:260-276.

66.   Allez M, Mayer L. Regulatory T cells: peace keepers in the gut. Inflamm Bowel Dis. 2004;10:666-676.

67.   Denning TL, Wang YC, Patel SR, et al. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. Nat Immunol. 2007;8:1086-1094.

68.   Hansen TS, Jess T, Vind I, et al. Environmental factors in inflammatory bowel disease: a case-control study based on a Danish inception cohort. J Crohns Colitis. 2011;5:577-584.

69.   Watson AJ, Hart AR. Environmental risk factors for inflammatory bowel disease: microbes, diet, and the appendix. Gastroenterology. 2011;141:768-770.

70.   Birrenbach T, Bocker U. Inflammatory bowel disease and smoking: a review of epidemiology, pathophysiology, and therapeutic implications. Inflamm Bowel Dis. 2004;10:848-859.

71.   Hviid A, Svanstrom H, Frisch M. Antibiotic use and inflammatory bowel diseases in childhood. Gut. 2011;60:49-54.

72.   Andersson RE, Olaison G, Tysk C, et al. Appendectomy and protection against ulcerative colitis. N Engl J Med. 2001;344:808-814.

73.   Rutgeerts P, D'Haens G, Hiele M, et al. Appendectomy protects against ulcerative colitis. Gastroenterology. 1994;106:1251-1253.

74.   Frisch M, Pedersen BV, Andersson RE. Appendicitis, mesenteric lymphadenitis, and subsequent risk of ulcerative colitis: cohort studies in Sweden and Denmark. BMJ. 2009;338:b716.

75.   Duggan AE, Usmani I, Neal KR, et al. Appendicectomy, childhood hygiene, Helicobacter pylori status, and risk of inflammatory bowel disease: a case control study. Gut. 1998;43:494-498.

76.   Russel MG, Dorant E, Brummer RJ, et al. Appendectomy and the risk of developing ulcerative colitis or Crohn's disease: results of a large case-control study. South Limburg Inflammatory Bowel Disease Study Group. Gastroenterology. 1997;113:377-382.

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

78.   Munkholm P, Langholz E, Davidsen M, et al. Frequency of glucocorticoid resistance and dependency in Crohn's disease. Gut. 1994;35:360-362.

79.   Dignass A, Van Assche G, Lindsay JO, et al. The second European evidence-based consensus on the diagnosis and management of Crohn´s disease: Current management. Journal of Crohn´s and Colitis. 2010;4:28-62.

80.   Hayashi R, Wada H, Ito K, et al. Effects of glucocorticoids on gene transcription. Eur J Pharmacol. 2004;500:51-62.

81.   De Iudicibus S, Franca R, Martelossi S, et al. Molecular mechanism of glucocorticoid resistance in inflammatory bowel disease. World J Gastroenterol. 2011;17:1095-1108.

82.   Farrell RJ, Kelleher D. Glucocorticoid resistance in inflammatory bowel disease. J Endocrinol. 2003;178:339-346.

83.   Azad Khan AK, Piris J, Truelove SC. An experiment to determine the active therapeutic moiety of sulphasalazine. Lancet. 1977;2:892-895.

84.   Klotz U, Maier K, Fischer C, et al. Therapeutic efficacy of sulfasalazine and its metabolites in patients with ulcerative colitis and Crohn's disease. N Engl J Med. 1980;303:1499-1502.

85.   Schroder H, Evans DA. Acetylator phenotype and adverse effects of sulphasalazine in healthy subjects. Gut. 1972;13:278-284.

86.   Loftus EV, Jr., Kane SV, Bjorkman D. Systematic review: short-term adverse effects of 5-aminosalicylic acid agents in the treatment of ulcerative colitis. Aliment Pharmacol Ther. 2004;19:179-189.

87.   Van Staa TP, Travis S, Leufkens HG, et al. 5-aminosalicylic acids and the risk of renal disease: a large British epidemiologic study. Gastroenterology. 2004;126:1733-1739.

88.   Travis SPL, Stange EF, Lemann M, et al. European evidence-based Consensus on the management of ulcerative colitis: Current management. Journal of Crohn´s and Colitis. 2008;2:24-62.

89.   Myers B, Evans DN, Rhodes J, et al. Metabolism and urinary excretion of 5-amino salicylic acid in healthy volunteers when given intravenously or released for absorption at different sites in the gastrointestinal tract. Gut. 1987;28:196-200.

90.   D'Haens G, Hommes D, Engels L, et al. Once daily MMX mesalazine for the treatment of mild-to-moderate ulcerative colitis: a phase II, dose-ranging study. Aliment Pharmacol Ther. 2006;24:1087-1097.

91.   Safdi AV, Cohen RD. Review article: increasing the dose of oral mesalazine therapy for active ulcerative colitis does not improve remission rates. Aliment Pharmacol Ther. 2007;26:1179-1186.

92.   Rousseaux C, Lefebvre B, Dubuquoy L, et al. Intestinal antiinflammatory effect of 5-aminosalicylic acid is dependent on peroxisome proliferator-activated receptor-gamma. J Exp Med. 2005;201:1205-1215.

93.   Desreumaux P, Ghosh S. Review article: mode of action and delivery of 5-aminosalicylic acid - new evidence. Aliment Pharmacol Ther. 2006;24 Suppl 1:2-9.

94.   Dubuquoy L, Dharancy S, Nutten S, et al. Role of peroxisome proliferator-activated receptor gamma and retinoid X receptor heterodimer in hepatogastroenterological diseases. Lancet. 2002;360:1410-1418.

95.   Stolfi C, Pellegrini R, Franze E, et al. Molecular basis of the potential of mesalazine to prevent colorectal cancer. World J Gastroenterol. 2008;14:4434-4439.

96.   Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut. 2001;48:526-535.

97.   Hanauer SB, Feagan BG, Lichtenstein GR, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet. 2002;359:1541-1549.

98.   Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med. 2005;353:2462-2476.

99.   Cassinotti A, Travis S. Incidence and clinical significance of immunogenicity to infliximab in Crohn's disease: a critical systematic review. Inflamm Bowel Dis. 2009;15:1264-1275.

100.   Hanauer SB, Sandborn WJ, Rutgeerts P, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. Gastroenterology. 2006;130:323-333.

101.   Sandborn WJ, Van Assche G, Reinisch W, et al. Adalimumab Induces and Maintains Clinical Remission in Patients With Moderate-to-Severe Ulcerative Colitis. Gastroenterology. 2012;142:257-265.

102.   Bendtzen K, Geborek P, Svenson M, et al. Individualized monitoring of drug bioavailability and immunogenicity in rheumatoid arthritis patients treated with the tumor necrosis factor alpha inhibitor infliximab. Arthritis Rheum. 2006;54:3782-3789.

103.   Breedveld FC, Weisman MH, Kavanaugh AF, et al. The PREMIER study: A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. Arthritis Rheum. 2006;54:26-37.

104.   Leonardi C, Langley RG, Papp K, et al. Adalimumab for treatment of moderate to severe chronic plaque psoriasis of the hands and feet: efficacy and safety results from REACH, a randomized, placebo-controlled, double-blind trial. Arch Dermatol. 2011;147:429-436.

105.   Maksymowych WP, Salonen D, Inman RD, et al. Low-dose infliximab (3 mg/kg) significantly reduces spinal inflammation on magnetic resonance imaging in patients with ankylosing spondylitis: a randomized placebo-controlled study. J Rheumatol. 2010;37:1728-1734.

106.   Reich K, Nestle FO, Papp K, et al. Improvement in quality of life with infliximab induction and maintenance therapy in patients with moderate-to-severe psoriasis: a randomized controlled trial. Br J Dermatol. 2006;154:1161-1168.

107.   van der Heijde D, Schiff MH, Sieper J, et al. Adalimumab effectiveness for the treatment of ankylosing spondylitis is maintained for up to 2 years: long-term results from the ATLAS trial. Ann Rheum Dis. 2009;68:922-929.

108.   Sandborn WJ, Feagan BG, Stoinov S, et al. Certolizumab pegol for the treatment of Crohn's disease. N Engl J Med. 2007;357:228-238.

109.   Moreland LW, Weinblatt ME, Keystone EC, et al. Etanercept treatment in adults with established rheumatoid arthritis: 7 years of clinical experience. J Rheumatol. 2006;33:854-861.

110.   Martin-Mola E, Sieper J, Leirisalo-Repo M, et al. Sustained efficacy and safety, including patient-reported outcomes, with etanercept treatment over 5 years in patients with ankylosing spondylitis. Clin Exp Rheumatol. 2010;28:238-245.

111.   Sandborn WJ, Hanauer SB, Katz S, et al. Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. Gastroenterology. 2001;121:1088-1094.

112.   Papadakis KA, Targan SR. Tumor necrosis factor: biology and therapeutic inhibitors. Gastroenterology. 2000;119:1148-1157.

113.   Braegger CP, Nicholls S, Murch SH, et al. Tumour necrosis factor alpha in stool as a marker of intestinal inflammation. Lancet. 1992;339:89-91.

114.   Murch SH, Lamkin VA, Savage MO, et al. Serum concentrations of tumour necrosis factor alpha in childhood chronic inflammatory bowel disease. Gut. 1991;32:913-917.

115.   Breese EJ, Michie CA, Nicholls SW, et al. Tumor necrosis factor alpha-producing cells in the intestinal mucosa of children with inflammatory bowel disease. Gastroenterology. 1994;106:1455-1466.

116.   Chowers Y, Sturm A, Sans M, et al. Report of the ECCO workshop on anti-TNF therapy failures in inflammatory bowel diseases: biological roles and effects of TNF and TNF antagonists. J Crohns Colitis. 2010;4:367-376.

117.   Mitoma H, Horiuchi T, Hatta N, et al. Infliximab induces potent anti-inflammatory responses by outside-to-inside signals through transmembrane TNF-alpha. Gastroenterology. 2005;128:376-392.

118.   Di Sabatino A, Ciccocioppo R, Cinque B, et al. Defective mucosal T cell death is sustainably reverted by infliximab in a caspase dependent pathway in Crohn's disease. Gut. 2004;53:70-77.

119.   Vassalli P. The pathophysiology of tumor necrosis factors. Annu Rev Immunol. 1992;10:411-452.

120.   Mitoma H, Horiuchi T, Tsukamoto H, et al. Mechanisms for cytotoxic effects of anti-tumor necrosis factor agents on transmembrane tumor necrosis factor alpha-expressing cells: comparison among infliximab, etanercept, and adalimumab. Arthritis Rheum. 2008;58:1248-1257.

121.   Scallon BJ, Moore MA, Trinh H, et al. Chimeric anti-TNF-alpha monoclonal antibody cA2 binds recombinant transmembrane TNF-alpha and activates immune effector functions. Cytokine. 1995;7:251-259.

122.   Kaymakcalan Z, Sakorafas P, Bose S, et al. Comparisons of affinities, avidities, and complement activation of adalimumab, infliximab, and etanercept in binding to soluble and membrane tumor necrosis factor. Clin Immunol. 2009;131:308-316.

123.   Lugering A, Schmidt M, Lugering N, et al. Infliximab induces apoptosis in monocytes from patients with chronic active Crohn's disease by using a caspase-dependent pathway. Gastroenterology. 2001;121:1145-1157.

124.   Shen C, Van Assche G, Colpaert S, et al. Adalimumab induces apoptosis of human monocytes: a comparative study with infliximab and etanercept. Aliment Pharmacol Ther. 2005;21:251-258.

125.   Van den Brande JM, Braat H, van den Brink GR, et al. Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. Gastroenterology. 2003;124:1774-1785.

126.   Boirivant M, Marini M, Di FG, et al. Lamina propria T cells in Crohn's disease and other gastrointestinal inflammation show defective CD2 pathway-induced apoptosis. Gastroenterology. 1999;116:557-565.

127.   Ina K, Itoh J, Fukushima K, et al. Resistance of Crohn's disease T cells to multiple apoptotic signals is associated with a Bcl-2/Bax mucosal imbalance. J Immunol. 1999;163:1081-1090.

128.   ten Hove T, van MC, Peppelenbosch MP, et al. Infliximab treatment induces apoptosis of lamina propria T lymphocytes in Crohn's disease. Gut. 2002;50:206-211.

129.   Vos AC, Wildenberg ME, Duijvestein M, et al. Anti-tumor necrosis factor-alpha antibodies induce regulatory macrophages in an Fc region-dependent manner. Gastroenterology. 2011;140:221-230.

130.   Veltkamp C, Anstaett M, Wahl K, et al. Apoptosis of regulatory T lymphocytes is increased in chronic inflammatory bowel disease and reversed by anti-TNFalpha treatment. Gut. 2011;60:1345-1353.

131.   Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med. 2009;361:2066-2078.

132.   Izcue A, Coombes JL, Powrie F. Regulatory lymphocytes and intestinal inflammation. Annu Rev Immunol. 2009;27:313-338.

133.   Maul J, Loddenkemper C, Mundt P, et al. Peripheral and intestinal regulatory CD4+ CD25(high) T cells in inflammatory bowel disease. Gastroenterology. 2005;128:1868-1878.

134.   Takahashi M, Nakamura K, Honda K, et al. An inverse correlation of human peripheral blood regulatory T cell frequency with the disease activity of ulcerative colitis. Dig Dis Sci. 2006;51:677-686.

135.   Satsangi J, Silverberg MS, Vermeire S, et al. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut. 2006;55:749-753.

136.   de Ridder L, Rings EH, Damen GM, et al. Infliximab dependency in pediatric Crohn's disease: long-term follow-up of an unselected cohort. Inflamm Bowel Dis. 2008;14:353-358.

137.   Faubion WA, Jr., Loftus EV, Jr., Harmsen WS, et al. The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study. Gastroenterology. 2001;121:255-260.

138.   Jakobsen C, Munkholm P, Paerregaard A, et al. Steroid dependency and pediatric inflammatory bowel disease in the era of immunomodulators-A population-based study. Inflamm Bowel Dis. 2010.

139.   Wewer V, Riis L, Vind I, et al. Infliximab dependency in a national cohort of children with Crohn's disease. J Pediatr Gastroenterol Nutr. 2006;42:40-45.

140.   Franchimont DP, Louis E, Croes F, et al. Clinical pattern of corticosteroid dependent Crohn's disease. Eur J Gastroenterol Hepatol. 1998;10:821-825.

141.   Hyams J, Markowitz J, Lerer T, et al. The natural history of corticosteroid therapy for ulcerative colitis in children. Clin Gastroenterol Hepatol. 2006;4:1118-1123.

142.   Reinisch W, Gasche C, Wyatt J, et al. Steroid dependency in Crohn's disease. Lancet. 1995;345:859.

143.   Tung J, Loftus EV, Jr., Freese DK, et al. A population-based study of the frequency of corticosteroid resistance and dependence in pediatric patients with Crohn's disease and ulcerative colitis. Inflamm Bowel Dis. 2006;12:1093-1100.

144.   Stange EF, Travis SP, Vermeire S, et al. European evidence based consensus on the diagnosis and management of Crohn's disease: definitions and diagnosis. Gut. 2006;55 Suppl 1:i1-15.

145.   Stange EF, Travis SPL, Vermeire S, et al. European evidence-based consensus on the diagnosis and management of ulcerative colitis: Definitions and diagnosis. Journal of Crohn´s and Colitis. 2008;2:1-23.

146.   Gower-Rousseau C, Dauchet L, Vernier-Massouille G, et al. The natural history of pediatric ulcerative colitis: a population-based cohort study. Am J Gastroenterol. 2009;104:2080-2088.

147.   Vernier-Massouille G, Balde M, Salleron J, et al. Natural history of pediatric Crohn's disease: a population-based cohort study. Gastroenterology. 2008;135:1106-1113.

148.   Chow DK, Sung JJ, Tsoi KK, et al. Predictors of corticosteroid-dependent and corticosteroid-refractory inflammatory bowel disease: analysis of a Chinese cohort study. Aliment Pharmacol Ther. 2009;29:843-854.

149.   Ho GT, Chiam P, Drummond H, et al. The efficacy of corticosteroid therapy in inflammatory bowel disease: analysis of a 5-year UK inception cohort. Aliment Pharmacol Ther. 2006;24:319-330.

150.   Papi C, Festa V, Leandro G, et al. Long-term outcome of Crohn's disease following corticosteroid-induced remission. Am J Gastroenterol. 2007;102:814-819.

151.   Hyams J, Crandall W, Kugathasan S, et al. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. Gastroenterology. 2007;132:863-873.

152.   Peyrin-Biroulet L, Deltenre P, de Suray N, et al. Efficacy and safety of tumor necrosis factor antagonists in Crohn's disease: meta-analysis of placebo-controlled trials. Clin Gastroenterol Hepatol. 2008;6:644-653.

153.   Present DH, Rutgeerts P, Targan S, et al. Infliximab for the treatment of fistulas in patients with Crohn's disease. N Engl J Med. 1999;340:1398-1405.

154.   de RL, Escher JC, Bouquet J, et al. Infliximab therapy in 30 patients with refractory pediatric crohn disease with and without fistulas in The Netherlands. J Pediatr Gastroenterol Nutr. 2004;39:46-52.

155.   Caspersen S, Elkjaer M, Riis L, et al. Infliximab for inflammatory bowel disease in Denmark 1999-2005: clinical outcome and follow-up evaluation of malignancy and mortality. Clin Gastroenterol Hepatol. 2008;6:1212-1217.

156.   Ljung T, Karlen P, Schmidt D, et al. Infliximab in inflammatory bowel disease: clinical outcome in a population based cohort from Stockholm County. Gut. 2004;53:849-853.

157.   Kaur N, Mahl TC. Pneumocystis jiroveci (carinii) pneumonia after infliximab therapy: a review of 84 cases. Dig Dis Sci. 2007;52:1481-1484.

158.   Keane J, Gershon S, Wise RP, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med. 2001;345:1098-1104.

159.   Lees CW, Ali AI, Thompson AI, et al. The safety profile of anti-tumour necrosis factor therapy in inflammatory bowel disease in clinical practice: analysis of 620 patient-years follow-up. Aliment Pharmacol Ther. 2009;29:286-297.

160. Mackey AC, Green L, Liang LC, et al. Hepatosplenic T cell lymphoma associated with infliximab use in young patients treated for inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 2007;44:265-267.

161. Warris A, Bjorneklett A, Gaustad P. Invasive pulmonary aspergillosis associated with infliximab therapy. N Engl J Med. 2001;344:1099-1100.

162. Bodger K, Kikuchi T, Hughes D. Cost-effectiveness of biological therapy for Crohn's disease: Markov cohort analyses incorporating United Kingdom patient-level cost data. Aliment Pharmacol Ther. 2009;30:265-274.

163. Saro C, da la Coba C, Casado MA, et al. Resource use in patients with Crohn's disease treated with infliximab. Aliment Pharmacol Ther. 2007;26:1313-1323.

164. Sutherland L, Macdonald JK. Oral 5-aminosalicylic acid for maintenance of remission in ulcerative colitis. Cochrane Database Syst Rev. 2006;CD000544.

165. Sutherland L, Macdonald JK. Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis. Cochrane Database Syst Rev. 2006;CD000543.

166. Lochs H, Mayer M, Fleig WE, et al. Prophylaxis of postoperative relapse in Crohn's disease with mesalamine: European Cooperative Crohn's Disease Study VI. Gastroenterology. 2000;118:264-273.

167. McLeod RS, Wolff BG, Steinhart AH, et al. Prophylactic mesalamine treatment decreases postoperative recurrence of Crohn's disease. Gastroenterology. 1995;109:404-413.

168. Modigliani R, Colombel JF, Dupas JL, et al. Mesalamine in Crohn's disease with steroid-induced remission: effect on steroid withdrawal and remission maintenance, Groupe d'Etudes Therapeutiques des Affections Inflammatoires Digestives. Gastroenterology. 1996;110:688-693.

169. Prantera C, Cottone M, Pallone F, et al. Mesalamine in the treatment of mild to moderate active Crohn's ileitis: results of a randomized, multicenter trial. Gastroenterology. 1999;116:521-526.

170. Singleton JW, Hanauer SB, Gitnick GL, et al. Mesalamine capsules for the treatment of active Crohn's disease: results of a 16-week trial. Pentasa Crohn's Disease Study Group. Gastroenterology. 1993;104:1293-1301.

171. Sutherland LR, Martin F, Bailey RJ, et al. A randomized, placebo-controlled, double-blind trial of mesalamine in the maintenance of remission of Crohn's disease. The Canadian

Mesalamine for Remission of Crohn's Disease Study Group. Gastroenterology. 1997;112:1069-1077.

172.   Akobeng AK, Gardener E. Oral 5-aminosalicylic acid for maintenance of medically-induced remission in Crohn's Disease. Cochrane Database Syst Rev. 2005;CD003715.

173.   Camma C, Giunta M, Rosselli M, et al. Mesalamine in the maintenance treatment of Crohn's disease: a meta-analysis adjusted for confounding variables. Gastroenterology. 1997;113:1465-1473.

174.   Hanauer SB, Stromberg U. Oral Pentasa in the treatment of active Crohn's disease: A meta-analysis of double-blind, placebo-controlled trials. Clin Gastroenterol Hepatol. 2004;2:379-388.

175.   Travis SP, Stange EF, Lemann M, et al. European evidence based consensus on the diagnosis and management of Crohn's disease: current management. Gut. 2006;55 Suppl 1:i16-i35.

176.   Silverstein MD, Loftus EV, Sandborn WJ, et al. Clinical course and costs of care for Crohn's disease: Markov model analysis of a population-based cohort. Gastroenterology. 1999;117:49-57.

177.   Caprilli R, Gassull MA, Escher JC, et al. European evidence based consensus on the diagnosis and management of Crohn's disease: special situations. Gut. 2006;55 Suppl 1:i36-i58.

178.   Ferrante M, Vermeire S, Fidder H, et al. Long-term outcome after infliximab for refractory ulcerative colitis. J Crohns Colitis. 2008;2:219-225.

179.   Oussalah A, Evesque L, Laharie D, et al. A multicenter experience with infliximab for ulcerative colitis: outcomes and predictors of response, optimization, colectomy, and hospitalization. Am J Gastroenterol. 2010;105:2617-2625.

180.   Schnitzler F, Fidder H, Ferrante M, et al. Long-term outcome of treatment with infliximab in 614 patients with Crohn's disease: results from a single-centre cohort. Gut. 2009;58:492-500.

181.   Billioud V, Sandborn WJ, Peyrin-Biroulet L. Loss of response and need for adalimumab dose intensification in Crohn's disease: a systematic review. Am J Gastroenterol. 2011;106:674-684.

182.   Gisbert JP, Panes J. Loss of response and requirement of infliximab dose intensification in Crohn's disease: a review. Am J Gastroenterol. 2009;104:760-767.

183.   Duricova D, Malickova K, Bortlik M, et al. Predictors of Sustained Response to Infliximab in Patients with Crohn´s Disease: A Single Cohort Study. Gastroenterology. 2011;140:P S-593.

184.   Karmiris K, Paintaud G, Noman M, et al. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. Gastroenterology. 2009;137:1628-1640.

185.   Maser EA, Villela R, Silverberg MS, et al. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. Clin Gastroenterol Hepatol. 2006;4:1248-1254.

186.   Seow CH, Newman A, Irwin SP, et al. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. Gut. 2010;59:49-54.

187.   Baert F, Noman M, Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med. 2003;348:601-608.

188.   Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med. 2010;362:1383-1395.

189.   Hanauer SB, Wagner CL, Bala M, et al. Incidence and importance of antibody responses to infliximab after maintenance or episodic treatment in Crohn's disease. Clin Gastroenterol Hepatol. 2004;2:542-553.

190.   Sandborn WJ, Hanauer SB, Rutgeerts P, et al. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. Gut. 2007;56:1232-1239.

191.   West RL, Zelinkova Z, Wolbink GJ, et al. Immunogenicity negatively influences the outcome of adalimumab treatment in Crohn's disease. Aliment Pharmacol Ther. 2008;28:1122-1126.

192.   Fasanmade AA, Adedokun OJ, Ford J, et al. Population pharmacokinetic analysis of infliximab in patients with ulcerative colitis. Eur J Clin Pharmacol. 2009;65:1211-1228.

193.   Malickova K, Bortlik M, Duricova D, et al. Vliv albuminemie na farmakokinetiku infliximabu u nemocnych s idiopatickymi strevnimi zanety. Gastroent Hepatol. 2011;65:70-74.

194.   Afif W, Loftus EV, Jr., Faubion WA, et al. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. Am J Gastroenterol. 2010;105:1133-1139.

195.	Bendtzen K, Ainsworth M, Steenholdt C, et al. Individual medicine in inflammatory bowel disease: monitoring bioavailability, pharmacokinetics and immunogenicity of anti-tumour necrosis factor-alpha antibodies. Scand J Gastroenterol. 2009;44:774-781.

196.	Colombel JF, Feagan BG, Sandborn WJ, et al. Therapeutic drug monitoring of biologics for inflammatory bowel disease. Inflamm Bowel Dis. 2012;18:349-358.

197.	Duricova D, Bortlik M, Komarek V, et al. Skin Complications During Therapy With Anti-TNFa Preparations: Experience of a Single Centre. Gastroenterology. 2010;138:P S-763.

198.	Rahier JF, Buche S, Peyrin-Biroulet L, et al. Severe skin lesions cause patients with inflammatory bowel disease to discontinue anti-tumor necrosis factor therapy. Clin Gastroenterol Hepatol. 2010;8:1048-1055.

199.	Van Moerkercke W, Ackaert C, Jurgens M, et al. Anti-TNFa Induced Severe Arthralgia as a manifestation of Autoimmunity? Gastroenterology. 2010;Supp1;138:S-60-S-61.

200.	Braun J, Baraliakos X, Listing J, et al. Differences in the incidence of flares or new onset of inflammatory bowel diseases in patients with ankylosing spondylitis exposed to therapy with anti-tumor necrosis factor alpha agents. Arthritis Rheum. 2007;57:639-647.

201.	Jakobsen C, Paerregaard A, Munkholm P, et al. Paediatric inflammatory bowel disease during a 44-year period in Copenhagen County: occurrence, course and prognosis--a population-based study from the Danish Crohn Colitis Database. Eur J Gastroenterol Hepatol. 2009;21:1291-1301.

202.	Van Limbergen J, Russell RK, Drummond HE, et al. Definition of phenotypic characteristics of childhood-onset inflammatory bowel disease. Gastroenterology. 2008;135:1114-1122.

203.	Kamm MA, Ng SC. Perianal fistulizing Crohn's disease: a call to action. Clin Gastroenterol Hepatol. 2008;6:7-10.

204.	Taylor KD, Plevy SE, Yang H, et al. ANCA pattern and LTA haplotype relationship to clinical responses to anti-TNF antibody treatment in Crohn's disease. Gastroenterology. 2001;120:1347-1355.

205.	Hlavaty T, Pierik M, Henckaerts L, et al. Polymorphisms in apoptosis genes predict response to infliximab therapy in luminal and fistulizing Crohn's disease. Aliment Pharmacol Ther. 2005;22:613-626.

206.   Gonzalez S, Rodrigo L, Martinez-Borra J, et al. TNF-alpha -308A promoter polymorphism is associated with enhanced TNF-alpha production and inflammatory activity in Crohn's patients with fistulizing disease. Am J Gastroenterol. 2003;98:1101-1106.

207.   Sykora J, Subrt I, Didek P, et al. Cytokine tumor necrosis factor-alpha A promoter gene polymorphism at position -308 G-->A and pediatric inflammatory bowel disease: implications in ulcerative colitis and Crohn's disease. J Pediatr Gastroenterol Nutr. 2006;42:479-487.

208.   Louis E, Vermeire S, Rutgeerts P, et al. A positive response to infliximab in Crohn disease: association with a higher systemic inflammation before treatment but not with -308 TNF gene polymorphism. Scand J Gastroenterol. 2002;37:818-824.

209.   Mascheretti S, Hampe J, Kuhbacher T, et al. Pharmacogenetic investigation of the TNF/TNF-receptor system in patients with chronic active Crohn's disease treated with infliximab. Pharmacogenomics J. 2002;2:127-136.

210.   Gupta N, Cohen SA, Bostrom AG, et al. Risk factors for initial surgery in pediatric patients with Crohn's disease. Gastroenterology. 2006;130:1069-1077.

211.   Rubenstein JH, Chong RY, Cohen RD. Infliximab decreases resource use among patients with Crohn's disease. J Clin Gastroenterol. 2002;35:151-156.

212.   Taxonera C, Rodrigo L, Casellas F, et al. Infliximab maintenance therapy is associated with decreases in direct resource use in patients with luminal or fistulizing Crohn's disease. J Clin Gastroenterol. 2009;43:950-956.

213.   Peyrin-Biroulet L, Oussalah A, Williet N, et al. Impact of azathioprine and tumour necrosis factor antagonists on the need for surgery in newly diagnosed Crohn's disease. Gut. 2011;60:930-936.

214.   Bebb JR, Scott BB. How effective are the usual treatments for Crohn's disease? Aliment Pharmacol Ther. 2004;20:151-159.

215.   Bokemeyer B, Katalinic A, Klugmann T, et al. Predictive factors for a mild course of Crohn´s disease. Journal of Crohn´s and Colitis. 2009;3:S82-S83.

216.   Kane SV, Cohen RD, Aikens JE, et al. Prevalence of nonadherence with maintenance mesalamine in quiescent ulcerative colitis. Am J Gastroenterol. 2001;96:2929-2933.

217.   Harris MS, Lichtenstein GR. Review article: delivery and efficacy of topical 5-aminosalicylic acid (mesalazine) therapy in the treatment of ulcerative colitis. Aliment Pharmacol Ther. 2011;33:996-1009.

218.	Lichtenstein GR, Kamm MA. Review article: 5-aminosalicylate formulations for the treatment of ulcerative colitis--methods of comparing release rates and delivery of 5-aminosalicylate to the colonic mucosa. Aliment Pharmacol Ther. 2008;28:663-673.

219.	De Iudicibus S, Stocco G, Martelossi S, et al. Association of BclI polymorphism of the glucocorticoid receptor gene locus with response to glucocorticoids in inflammatory bowel disease. Gut. 2007;56:1319-1320.

220.	Louis E, Peeters M, Franchimont D, et al. Tumour necrosis factor (TNF) gene polymorphism in Crohn's disease (CD): influence on disease behaviour? Clin Exp Immunol. 2000;119:64-68.

221.	Cucchiara S, Latiano A, Palmieri O, et al. Polymorphisms of tumor necrosis factor-alpha but not MDR1 influence response to medical therapy in pediatric-onset inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 2007;44:171-179.

222.	Farrell RJ, Murphy A, Long A, et al. High multidrug resistance (P-glycoprotein 170) expression in inflammatory bowel disease patients who fail medical therapy. Gastroenterology. 2000;118:279-288.

223.	Hirano T, Onda K, Toma T, et al. MDR1 mRNA expressions in peripheral blood mononuclear cells of patients with ulcerative colitis in relation to glucocorticoid administration. J Clin Pharmacol. 2004;44:481-486.

224.	Palmieri O, Latiano A, Valvano R, et al. Multidrug resistance 1 gene polymorphisms are not associated with inflammatory bowel disease and response to therapy in Italian patients. Aliment Pharmacol Ther. 2005;22:1129-1138.

225.	Potocnik U, Ferkolj I, Glavac D, et al. Polymorphisms in multidrug resistance 1 (MDR1) gene are associated with refractory Crohn disease and ulcerative colitis. Genes Immun. 2004;5:530-539.

226.	Markowitz J, Hyams J, Mack D, et al. Corticosteroid therapy in the age of infliximab: acute and 1-year outcomes in newly diagnosed children with Crohn's disease. Clin Gastroenterol Hepatol. 2006;4:1124-1129.

227.	Rutgeerts P, Feagan BG, Lichtenstein GR, et al. Comparison of scheduled and episodic treatment strategies of infliximab in Crohn's disease. Gastroenterology. 2004;126:402-413.

228.	Fidder H, Schnitzler F, Ferrante M, et al. Long-term safety of infliximab for the treatment of inflammatory bowel disease: a single-centre cohort study. Gut. 2009;58:501-508.

229.	Fiorino G, Allez M, Malesci A, et al. Review article: anti TNF-alpha induced psoriasis in patients with inflammatory bowel disease. Aliment Pharmacol Ther. 2009;29:921-927.

230.	Robinson A, Thompson DG, Wilkin D, et al. Guided self-management and patient-directed follow-up of ulcerative colitis: a randomised trial. Lancet. 2001;358:976-981.

231.	Robinson A. Review article: improving adherence to medication in patients with inflammatory bowel disease. Aliment Pharmacol Ther. 2008;27 Suppl 1:9-14.

APPENDIX: Papers I-IV