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AETIOLOGY AND PROGNOSIS OF PAEDIATRIC INFLAMMATORY BOWEL DISEASE

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Stockholm 2014

Published by Karolinska Institutet. Printed by Åtta.45 Tryckeri AB.

ISBN 978-91-7549-461-6

”Det finns saker, som man måste vara fackman för att inte förstå.”

Hjalmar Söderberg

To my family and those who are not - and yet are close to me and my ideas.

To all children with IBD.

ABSTRACT
The incidence rates of childhood-onset inflammatory bowel disease (IBD) have increased worldwide during recent decades. The changing incidence rates of paediatric IBD underscore the importance of early environmental exposures in the pathogenesis of the disease. It is possible that atypical bowel colonisation early in life creates less stable homeostasis between the host immune system and microbiota and thus might increase risk of CD later in life. Some recent studies have reported that the childhood-onset IBD phenotype is characterised by increasing intestinal involvement over time and rapid progression to complicated disease behaviour.

The aims of this thesis were to study trends in paediatric IBD incidence, to test if markers of atypical or disturbed early bowel colonisation are associated with an increased risk of CD and to describe the prognosis of childhood-onset IBD.

In **paper I** we conducted a follow-up study of the incidence of paediatric IBD in the general population-based catchment area of northern Stockholm County 2002-2007. Medical records of all 133 children diagnosed with IBD were scrutinised. The sex- and age-standardised incidence of paediatric IBD was 12.8 per 10^5 person-years. We concluded that the incidence of paediatric IBD during the study period was significantly higher than that observed in our earlier study covering 1990-2001.

In **paper II** we studied inpatient treatment for diagnoses associated with use of antibiotics and risk of CD. Patients with CD born 1973-1997 and matched controls were identified through Swedish population registers. Inpatient treatment for pneumonia before 5 years of age was associated with increased risk for CD (OR 3.54, 95% CI 1.78–7.04). We concluded that pneumonia, and thus antibiotic therapy, early in life was associated with subsequent CD risk and this may represent either causation or susceptibility.

In **paper III** we studied perinatal exposures and risk of CD during childhood. Patients diagnosed with paediatric CD 1990-2006 and matched controls and their perinatal exposures were identified through Swedish population registers. Birth by caesarean section was associated with a modestly increased risk for paediatric CD among boys (OR 1.25, 95% CI 1.01–1.54). We concluded that perinatal exposures associated with delivery mode may have a modest influence on CD risk during childhood among boys.

In **paper IV** we described the prognosis for all 280 patients with childhood-onset IBD in northern Stockholm County 1990-2007 over a median follow-up time of 8.8 years. From patient records we demonstrated that the cohort was characterised by extensive colitis that was relatively stable over time and associated with a relatively low risk of complications and intra-abdominal surgery. In conclusion, our findings confirm that patients with paediatric IBD have more widespread disease, but question the proposed dynamic and aggressive nature of the childhood-onset IBD phenotype.

This thesis adds to our knowledge about the incidence and risks for paediatric IBD and the prognosis of childhood-onset IBD. This information can be used as a foundation for discussions on future research in the field.
Keywords: inflammatory bowel disease, ulcerative colitis, Crohn's disease, risk factors, caesarean section, incidence, prognosis

LIST OF PUBLICATIONS

I. Malmborg P, Grahnquist L, Lindholm J, Montgomery S, Hildebrand H. Incidence of paediatric inflammatory bowel disease in northern Stockholm County 2002-2007. J Pediatr Gastroenterol Nutr. 2013

II. Hildebrand H, Malmborg P, Askling J, Ekbom A, Montgomery SM. Early life exposures associated with antibiotic use and risk of subsequent Crohn's disease. Scand J Gastroenterol. 2008;43(8):961-966

III. Malmborg P, Bahmanyar S, Grahnquist L, Hildebrand H, Montgomery S. Cesarean section and the risk of pediatric Crohn's disease. Inflamm Bowel Dis. 2012;18(4):703-8.

IV. Malmborg P, Grahnquist L, Ideström M, Lindholm J, Befrits R, Björk J, Montgomery S, Hildebrand H. Presentation and progression of childhood onset inflammatory bowel disease in northern Stockholm County. In manuscript.

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1 FOREWORD

From the very first day as paediatrician I started working in the field of paediatric gastroenterology. I have never regretted this as I find that paediatric gastroenterology covers most aspects of clinical medicine. The spectrum goes from intensive care of the newborn child with intestinal failure to consultation with the adolescent suffering from long-lasting stomach pain – a clinical practice that demands knowledge and judgement on how to manage both soma and psyche.

Most children with IBD do well most of the time. During recent decades, substantial progress has been made in the care of children with IBD. The development of protein-based drugs and minimally invasive surgery have revolutionised the treatment of children with severe forms of IBD. Nevertheless, these treatments sometimes have severe short- or long-term side effects and there are still forms of IBD that are resistant even to today's treatments. There is thus an urgent need for better treatments that could allow all children burdened by IBD to have a symptom-free childhood. It is my hope and belief that in the close future we will have unravelled the pathogenesis of IBD and thus be able to offer even the most chronically ill children treatments that make life easier.

Stockholm, February 2014

Petter Malmborg

2 LIST OF ABBREVIATIONS

anti-TNF	anti tumour necrosis factor
5-ASA	5-aminosalicylic acid
CD	Crohn's disease
CI	Confidence interval
DM1	Diabetes Mellitus type 1
IBD	Inflammatory bowel disease
IBDU	Inflammatory bowel disease unclassified
IM	Immunomodulators
OR	Odds ratio
UC	Ulcerative colitis

3 INTRODUCTION

The aetiology of inflammatory bowel disease (IBD) is still not clear. Genetic studies have demonstrated associations between IBD and genes that are involved in the immune system's recognition and handling of intestinal microbiota. The human large bowel and caecum are colonised at birth by microbiota (mostly bacteria and fungi) that help the host extract energy from carbohydrates that cannot be digested in the small bowel. The immune system's default reaction towards the microbiota is aggressive defence and inflammation. This primitive reaction needs to be balanced by anti-inflammatory mechanisms so that homeostasis between host and microbiota develops and is maintained. IBD is thought to be explained by a loss of the, during early life developed, tolerance to intestinal microbiota. The incidence of IBD (and other immune mediated inflammatory diseases) has increased dramatically during the last century. Living conditions and material circumstances in most countries worldwide have changed fundamentally following the industrial revolution. It is possible that the IBD epidemic is caused by a modern lifestyle that has rapidly altered our finely-tuned relationship (that has evolved over millions of years) with the microbiota we host.

4 BACKGROUND

4.1 HISTORICAL REMARKS

The name ulcerative colitis (UC) to describe a condition of chronic intestinal inflammation was already in use during the late 19th century (1, 2). Although several case reports (3-5) had been published describing chronic inflammation of the ileum, it was not until 1932, when Crohn, Ginzburg and Oppenheimer presented their case series on 14 patients with "regional enteritis", that the disease entity gained general recognition (6). The name of the disease entity was later abandoned in favour of the eponym "Crohn's disease" (CD) as by this time it had become obvious that the inflammation seen in "regional enteritis" did not have to involve the terminal ileum and could afflict any part of the gastrointestinal tract (7, 8).

4.2 DEFINITIONS AND DIAGNOSIS

CD and UC are characterised by chronic intestinal inflammation with specific but sometimes overlapping clinical, macroscopic and microscopic characteristics. In some ways CD and UC could be considered as the two extremes of a spectrum of chronic gut inflammation. As the two diseases share risk factors, frequently present with the same symptoms and signs and respond to the same treatments, they are often referred to under the common name inflammatory bowel disease (IBD).

There are as yet no internationally accepted criteria for the diagnosis of CD or UC. The current expert view is that the diagnoses should be established on the basis of non-strictly defined combinations of clinical presentation, macroscopic appearance (endoscopy and radiology) and microscopic findings with the caveat that differential diagnoses – including infections – should be excluded (9-15).

It is recommended that an IBD-colitis that cannot be classified as either CD or UC should be labelled inflammatory bowel disease unclassified (IBDU) until further discriminatory information can be obtained. If there is still no convincing evidence, even after thorough histopathological examination of the bowel after colectomy, use of the term indeterminate colitis (IC) is recommended (11).

The Porto criteria from 2005 are consensus recommendations from the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) on examinations for the diagnosis of paediatric IBD (15). These state that all children with suspected IBD should be examined with both gastro-duodenoscopy and ileocolonoscopy and that all children with suspected CD or IBDU also should be examined with imaging of the small bowel.

IBD is characterised by a chronically relapsing and remitting inflammation of the gastrointestinal tract. Patients with CD are typically burdened by a segmental and transmural intestinal inflammation that can involve all parts of the gastrointestinal tract.

In UC the intestinal inflammation is continuous and primarily superficial and restricted to the rectum and a variable extent of the colon in continuity.

Patients with UC are almost always troubled by bloody diarrhoea, abdominal cramps and urgency of defecation. Symptoms of CD vary depending on the location of the intestinal inflammation. CD patients with colonic involvement most often present with diarrhoea that may be bloody. Inflammation restricted to the distal ileum can produce only mild abdominal pains but may at the same time cause significant growth retardation and delayed puberty onset in children and adolescents (16). In some patients with CD, the major symptoms can be caused by perianal disease (fistulas, fissures and abscesses). Concomitant systemic symptoms such as fever and weight loss are much more common among patients with CD than with UC. Both diseases are sometimes accompanied by extra-intestinal manifestations involving joints, skin, liver and eyes.

In UC, microscopical examination often reveals pronounced distortions of crypt architecture that are accompanied by congested capillaries, goblet cell depletion and basal plasmocytosis as specific histopathological inflammatory characteristics. The inflammatory reaction in CD is characterised by discontinuous focal or patchy transmural inflammation, with epitheloid granulomas, that can induce fissuring and produce fibrosis.

All the characteristics of UC can also be caused by CD (although microscopic inflammation limited to the mucosa is very rarely seen in CD). Conversely, several CD manifestations exclude UC as a possible differential diagnosis. This explains why most follow-up studies report that a significant proportion of patients with UC (or IBDU) will ultimately be re-classified as having CD (17, 18).

Clinical, endoscopic and microscopic features helpful in distinguishing UC from CD are presented in **Table 1, 2 and 3.**

Table 1

Clinical features distinguishing UC from CD

Typical for UC
Bloody diarrhoea
Abdominal cramps
Urgency of defecation
Less common (and suggestive of CD), but compatible with UC
Weight loss*
Growth retardation**
Delayed puberty**
Incompatible with UC (and definite for CD)
Non-bloody diarrhoea
* Only seen in patients with severe gastrointestinal symptoms of UC. ** Only seen in children with long standing severe gastrointestinal symptoms of UC.

Modified from (9, 19)

Table 2

Endoscopic features distinguishing UC from CD

Typical for UC
Diffuse and continuous inflammation beginning in rectum and extending proximally to a variable extent; features of inflammation may include the following:
Granularity
Loss of vascular pattern
Friability
Small superficial ulcers in a background of diffuse inflammation
Mucopurulent exudates
Line of demarcation–an abrupt transition between abnormal and normal colonic mucosa
Less common (and suggestive of CD), but compatible with UC
Erythemateous inflammation of terminal ileum*
Erythemateous inflammation in gastric or duodenal mucosa
Perianal fissures
Oral ulcers
Incompatible with UC (and definite for CD)
Discontinuous intestinal inflammation**
Ulceration in terminal ileum
Ulceration in gastric or duodenal mucosa
Linear ulceration
Cobble stoning
Strictures
Fistulas
* Compatible with UC only when in conjunction with an active pancolitis (backwash ileitis) ** A relative macroscopic inflammatory rectal sparing can be seen in children with UC. A peri-appendiceal inflammation can be seen in patients with distal UC.

Modified from (9-11, 15, 19, 20).

Table 3

Microscopic features distinguishing UC from CD

Typical for UC
Chronic or chronic active colitis beginning in rectum and extending proximally to a variable extent; features of inflammation may include the following:
Goblet cell depletion
Distal Paneth cell metaplasia
Basal lymphoplasmacytosis
Crypt architectural distortion
Less common (and suggestive of CD), but compatible with UC
Microscopic ileitis without granuloma
Microscopic duodeno-gastritis without granuloma
Relative rectal sparing (histological inflammation less severe in rectum)*
Patchiness (varying intensity of colonic inflammation)*
Transmural inflammation (in severe attacks of UC)
Incompatible with UC (and definite for CD)
True (non-pericrypt) granulomas
Transmural lymphoid aggregates
Fissuring ulceration
* Microscopic relative rectal sparing and patchy colitis are more often seen in children with new-onset UC but can also be seen in adult patients with UC due to treatment.

Modified from (9-11, 15, 19, 20).

4.3 **CLASSIFICATION**

Inflammatory bowel diseases (IBD) are disorders of multifactorial cause that can present in many different ways and the disease burden throughout life can vary considerably from one patient to another.

The observed associations of certain early disease characteristics with a more severe disease course have influenced the creation and development of IBD classification systems. The subdivision of IBD patients in these phenotypes allows for more specific risk assessment that can be used to guide the choice and timing of medical and surgical treatment for the individual patient. The creation of international IBD classification systems has also enabled more valid comparisons of disease characteristics over time and between regions and populations. This has facilitated the quest for genetic traits and environmental exposures associated with certain subtypes of IBD.

The most important classification when trying to predict the disease course in an IBD patient is still the categorisation into CD or UC. Although there is a huge span in disease burden within both entities there are distinct group differences in prognosis between patients with CD and UC. The majority of UC patients can expect to face a quiescent disease course with little impact on life prospects (21, 22). However, with the diagnosis comes a risk of an acute bout of severe colitis that might demand a life-saving colectomy and an increased hazard of developing colorectal cancer (23). Thanks to modern treatment and follow-up strategies, patients diagnosed with UC in developed countries now seem to have an expected life-span equal to that of the general population (24). Patients diagnosed with CD can also expect to spend more time in remission than burdened by symptoms during the first decade after diagnosis (25, 26). Nonetheless, the potential of the intestinal inflammation in CD to produce fibrosis and fistulas explains why the majority of patients with this diagnosis eventually seem to require surgical treatment (27). Moreover, children diagnosed with CD (in contrast with children with UC) also face a substantial risk of growth retardation and delayed pubertal onset (16). It is thus not surprising that comparative studies have demonstrated that CD patients in general have lower self-reported quality of life than UC patients (28). Most contemporary studies still report a shorter life expectancy among CD patients, which primarily seems to be explained by progressive intestinal complications in a minority of patients (29, 30). Some recent studies have not been able to demonstrate that patients with CD have a mortality rate that differs significantly from the general population (31, 32).

The first paper to demonstrate that certain initial disease characteristics of CD have bearing on the future disease course was published in 1975 by Farmer et al, and provided evidence of the influence of anatomical disease location on prognosis (33). This landmark study was the foundation for the first international CD classification system, which was formed in Rome in 1988 (34). It was agreed that the anatomic location (33) and the clinical behaviour (35) were important predictive variables to include in the classification of CD patients . The Rome classification was revised and simplified in Vienna 1998 (36) when age at onset of disease (< 40 or ≥40 years at

diagnosis) was added (37). The classification of Montreal in 2005 modified the Vienna CD classification by also recognising childhood onset IBD as a separate category (< 17 years at diagnosis) (38). The Montreal classification moreover acknowledged that perianal disease should be classified separately from internal fistulising behaviour (39) and allowed upper gastrointestinal disease location to coexist with more distal disease. The Montreal consensus also for the first time applied a classification of UC, based on the extension of colonic inflammation and the severity at presentation or relapse, as studies had demonstrated that these characteristics are associated with risk of colectomy and colorectal cancer (22, 23).

The Paris classification (**Table 4**) from 2010 is a paediatric modification of the Montreal classification (40). As an adaption to paediatric practice, the presence or absence of growth failure during childhood was added as a discriminatory phenotype characteristic. A sub-division of childhood-onset IBD was proposed according to diagnosis before or after 10 years of age (41). The Paris classification also introduced a division of upper gastrointestinal disease in jejunal versus oesophago-gastro-duodenal disease (42). Subdivision of extensive colitis was also suggested to specify whether the inflammation extended proximal to the right colonic flexure. The possibility of both stenosing and penetrating disease behaviour co-existing in a patient created an option to sub-group patients (43).

Although already mentioned in the Vienna classification, the Paris classification stresses that the phenotyping should be based on the maximum disease location/extension ever observed and that the demarcation of the disease territory should be guided by observed inflammation at endoscopy or imaging and not by microscopic involvement.

There have been attempts to reclassify IBD based on genetic, serological or immunological findings rather than clinical characteristics (44). However, these more modern classifications have not yet been proven to be superior to the old ones in predicting disease course (45).

Table 4

Paris phenotype classification of childhood-onset IBD

Age		
	A1a	<10 years
	A1b	10-<17 years
	A2	17-<40 years
Growth retardation		
	G0	No growth retardation
	G1	Growth retardation
Ulcerative colitis		
Extension	E1	Ulcerative proctitis
	E2	Left sided UC (distal to splenic flexure)
	E3	Extensive UC (distal to hepatic flexure)
	E4	Pancolitis (proximal to hepatic flexure)
Crohn's disease		
Location	L1	Distal ileum ± limited caecal disease
	L2	Colonic disease
	L3	Ileocolonic disease
	L4a*	Upper GI disease, proximal to ligament of Treitz
	L4b*	Upper GI disease, distal to ligament of Treitz and proximal to distal ileum
Behaviour	B1	Non-stricturing, non-penetrating disease
	B2	Stricturing disease
	B3	Penetrating disease
	B2B3	Both stricturing and penetrating disease
	P**	Perianal disease

*L4a and L4b are modifiers that can be added to L1–L3 when concomitant upper gastrointestinal disease is present

**P is added to B1–B3 when concomitant perianal disease is present.

4.4 **AETIOLOGY**

The cause of IBD is yet not clear although available evidence supports the hypothesis that IBD results from a loss of the during early life developed immunological tolerance towards the intestinal microbiota and that this loss is a consequence of environmental exposures (as yet largely unidentified) among genetically susceptible individuals.

This hypothesis is constructed from data that to a large extent have been gleaned from epidemiological association studies.

4.4.1 Associations with infectious agents

The macro- and microscopic findings in CD resemble intestinal mycobacterial infections (46) and similarities in the presentation of UC and bacterial colitis (47) have spurred researchers to try to find infectious agents that could explain both diseases. Over the years many candidates – both bacterial (48, 49) and viral – (50, 51) have been associated with IBD. However, causal relationships have not been confirmed in treatment studies (52) and repeated histopathological and microbiological studies have for most agents failed to reproduce associations.

4.4.2 Associations with genes and family histories

Twin studies have stressed the importance of both genetic and environmental factors in the development of both UC and CD (53, 54). It might be assumed that the genetic influence on the pathogenesis is greater for patients that develop IBD during childhood, as they have been confronted with fewer exposures during their short life span. Studies on children that have developed IBD during infancy have also demonstrated that mutations in genes involved in the IL-10 signalling system seem to be the cause for the intestinal inflammation in rare cases (55).

The genome-wide association technique has revolutionised the possibility of finding links between phenotype and genotype. Somewhat more than 100 genes have now been shown to be significantly more common among IBD patients than in the general population (56). Approximately one-third of these loci confer susceptibility to both CD and UC (which is congruent with earlier studies that have demonstrated increased risk for CD among relatives to patients with UC and vice versa). The protein products of some of these genes have furthered our understanding of IBD pathogenesis. Some of the genes associated with CD translate to proteins that are involved in the innate immune system's microbial recognition (57) and autophagy (58). UC patients more often carries risk genes that are translated into proteins that are involved in the barrier function of the intestinal mucosal (59).

4.4.3 Associations with geography and period

There are considerable differences in the incidence of IBD across different geographic regions and over time (60). There is almost always a strong relationship between the occurrence of UC and CD. Populations with a high incidence of UC also have a high incidence of CD and vice versa (60). The highest incidence rates of IBD have been

observed in Western Europe and North America where studies exploring temporal trends indicate that these rates are still increasing (61-63). Several recent studies have demonstrated that the incidence of IBD is also increasing in former low-incidence regions such as Asia (64). Thus IBD seems to be an emerging global disease (60). Most incidence studies in adult populations show higher incidence of UC than CD (60). A consistent historical temporal pattern of a rising incidence of UC, followed by an increase in CD incidence approximately 15 to 20 years later, have been observed in high IBD incidence regions (65-68).

Rising incidence rates of childhood-onset IBD have also been observed worldwide (in both high and low income countries) during the last two decades (69). IBD can have its onset at all ages but the incidence peak is in young adulthood. It has been suggested that the increase in incidence of paediatric IBD could be explained by a trend towards the disease debuting at a younger age. A nationwide study from Switzerland that analysed IBD incidence in all ages found, on the contrary, a trend for disease onset occurring at an older age today as compared with recent decades (70). The increasing incidence of paediatric IBD in most countries seems to be due to a rising incidence of CD. The highest incidence rates of childhood-onset IBD have been observed in the Scandinavian countries and Canada (63, 71-73). A steep increase in paediatric IBD incidence and a shift in presentation from UC to CD was observed in northern Stockholm County in 1990-2001 (71).

4.4.4 Associations with ethnicity and socioeconomic position

A consistent finding in early observational studies was the association between high socioeconomic position and increased risks for both UC and CD (74-76). Although significant associations with socioeconomic position have been reproduced even in recent studies from high incidence areas (77), most studies have failed to demonstrate such an association (78, 79), indicating that modern living conditions might have reduced differences in certain environmental exposures that were formerly associated with socioeconomic position.

Several studies have demonstrated that different ethnic groups living in the same region may have significantly different IBD incidence rates (80). Whether this is explained by differences in genetic susceptibility or by differences in environmental exposures associated with ethnicity has been a source for debate. Second-generation immigrants to Great Britain, from Asian countries with low IBD incidence, seem to have a higher risk for IBD than both their parents and the indigenous European population (81); this indicates that environmental factors, not genetics, are the major driving force in changes of disease risk, at least in high incidence regions.

4.4.5 Association with early environmental exposures

The increasing incidence of paediatric IBD indicates that early environmental exposures are involved in the aetiology of the diseases. Known risk factors for adult-onset CD such as smoking (82) and use of oral hormonal contraceptive (83) would be expected to have little or no influence on the incidence of childhood-onset IBD. Perinatal (84) and childhood infections (85) have been associated with risk of IBD later

in life, but the global inverse relation between infant mortality (which is most often caused by infections) and IBD incidence argues against early infections as an important cause for the present epidemic of paediatric IBD. The protective effect of breast feeding has been demonstrated in several studies (86) but sheds no light on the enigma of rising childhood-onset IBD incidence, as breast feeding has increased in high incidence regions like Scandinavia during the last decades (87).

The hypothesis that the increasing incidence of immune mediated diseases in industrialised countries comes from altered relationships with the microbiota in early life (88, 89) has provided a new framework for interpretation of how early life exposures may influence future IBD risk. In this context, exposures that change or disturb the colonisation of the bowel after birth might create a less stable homeostasis between immune system and bowel flora and thus increase risk for later IBD. This hypothesis gains some credence from a study of siblings and IBD that found an association between having a younger sibling and a reduced CD risk, consistent with repeated re-colonisation of the bowel with an infantile flora from the younger sibling, stimulating appropriate immune system maturation in the elder sibling (90).

4.5 PATHOGENESIS

4.5.1 Early bacterial colonisation and the intestinal immune system

Mammalian young are normally confronted with appreciable numbers of microbiota for the very first time when passing through the birth canal during delivery. In humans, and other mammalian species that harbour large amounts of bacteria in the lower gastrointestinal tract, the normal colonisation of the bowel thus starts with faecal microbes from the mother. From an evolutionary perspective the importance of the caecal- and colonic microbiota comes from their capacity to produce energy-rich short chain fatty acids from saccharides (fibre) in the diet, which the host is unable to digest in the small intestine (91). The commensal microbiota thus improves the host's extraction of energy from the diet and also protects the host from pathogenic strains by competition for substrate and space (91). To allow this interplay, an evolutionary adaption of the host immune system must have taken place, allowing development of tolerance between commensal microbiota and host but yet maintaining an aggressive response to pathogenic microorganisms (92, 93).

The gastrointestinal tract is the primary site of interaction between the host immune system and microorganisms. Invading microorganisms are detected by the mucosal innate immune system, which responds with activation of inflammatory responses (94). If the host is to develop tolerance to the microbiota, the primitive non-selective inflammatory response of the innate immune system has to be balanced by down-regulation of the adaptive immune system.

The pathogenesis of IBD involves an inappropriate immunological response to bowel microbiota. This has been demonstrated in studies showing that colonic inflammation in patients with CD heals when a diverting ileo-stoma is constructed but recurs after re-anastomosis and re-establishment of the faecal stream (95). Genetic studies have widened our understanding of this inappropriate response by showing that failure of the

innate immune system to identify and handle luminal bacteria is associated with an increased risk for IBD (57, 58). It is believed that impaired control of the microbiota by the innate immune system may result in a compensatory but less balanced response from the adaptive immune system (96). In this model, certain environmental exposures might trigger dysfunctional immune responses in genetically susceptible individuals, responses that involve an inappropriate reaction to the commensal microbiota and thus cause chronic intestinal inflammation.

After birth, microbial colonisation of the bowel in the healthy child is dependent on hygiene and feeding practices (91) and the maturation of bowel flora diversity is influenced by the intestinal immune response of the host (93, 97). Significant differences in the composition of bowel flora have been demonstrated between Swedish infants born by the end of the 20th century and in preceding decades (98). Comparisons between children raised in an industrialised lifestyle in Europe and children living in rural Burkina Faso in West Africa have demonstrated profoundly greater gut microbial diversity and lower quantities of potentially pathogenic strains in the latter group (91).

While the immune system shapes the microbiota, it is also true that certain bacterial strains seem to have the capacity to modulate mucosal and systemic immune function of the host (99). However, there seems to be a rather narrow time window for establishing persistent bacterial colonisation of the bowel, as demonstrated in studies showing that organisms introduced during infancy may establish chronic persistence, whereas the same organisms are promptly cleared if first encountered later during childhood (100, 101). Consistent with this, it has been demonstrated that mode of delivery and early antibiotic treatment have an impact on the composition of the intestinal microbiota many years after the exposure (102, 103). Atypical or disturbed early colonisation of the bowel might thus hinder the establishment of appropriate microorganisms, required for normal development of homeostasis between microbiota and immune system, and may allow pathogens to persist. Abnormal microbial colonisation of the gut in early life might thus have implications for the risk of developing immune mediated diseases in general and IBD specifically later in life (93).

4.6 TREATMENT, PROGNOSIS AND PREDICTION

5-aminosalicylic acid (5-ASA) compounds were the first drugs that could be demonstrated to have an effect on the symptoms caused by IBD (104, 105). The introduction of corticosteroids during the 1950s provided potent anti-inflammatory drugs that radically reduced mortality owing to acute colitis in patients with UC (106). The experience that most patients who were saved from colectomy by corticosteroids during an attack of severe colitis nevertheless eventually required surgery (107) and that most patients with CD, despite pharmacological treatment, developed complications (strictures and fistulas) (27) led to the somewhat defeatist conclusion that medical treatments were probably unable to alter the natural disease course. The introduction of immunomodulators (IM) during the 1990s and inhibitors of tumour necrosis factor (anti-TNF) during first decade of the new millennium have evoked

hopes that modern treatments might have the potential to prevent development of disease complications in CD (108).

Population-based follow-up studies have shown that most patients with UC have a mild disease course with relatively little influence on everyday life (22). However, within the first decade after diagnosis, almost all patients with UC seem to be confronted with a relapse of symptomatic disease and roughly one-fourth of the patients will need a colectomy (22, 109). The surgery rate for UC patients seems not to have changed after the widespread introduction of immunosuppressive drugs (109). Colorectal cancer rates have decreased significantly over time, and a recent study from Denmark reported that a diagnosis of UC (or CD) no longer seems to significantly increase patients' risk of colorectal cancer (110). Prognostic studies of adult patients with CD have concluded that most patients at any given time during the first decade after diagnosis are fully capable of working (26). Nevertheless, data from referral centres (that tend to overestimate risks) indicate that a high proportion of patients with CD will in time develop stricturing or perforating complications that demand surgical treatment (27). There are conflicting results about whether the surgery rates have decreased among CD patients during the era of immunosuppressive therapy (109, 111).

Clinical experience suggests that there are several differences between childhood- and adult-onset IBD patients (112). Patients that have acquired UC during childhood seem to have a significantly higher risk of developing colorectal cancer later in life (23). Comparisons of phenotype characteristics at presentation and during short term follow-up suggest that childhood-onset IBD is characterised by more extensive intestinal involvement at diagnosis and rapid progression to extended disease and complicated disease behaviour (61, 113, 114). There are only a few population-based studies that more specifically describe the long term prognosis of patients with childhood-onset IBD (115-118). The reported substantial risks for corticosteroid dependency and surgery in some of these studies (116, 117) have lent further support to the hypothesis that childhood-onset IBD represents a more severe phenotype.

As paediatric IBD treatment also includes promotion of normal growth, aggressive therapy is sometimes advocated in children, even in the absence of gastrointestinal symptoms (119). The intensity of treatment for adult patients with IBD has been guided by the actual symptom burden and has aimed at establishing clinical remission (120). This paradigm has now been questioned in CD where (in contrast with patients with UC (121)) there is a weak relationship between intestinal mucosal inflammation and symptoms measured as disease activity indices (122). It has been suggested that the medical treatment should rather aim at promoting mucosal healing as this outcome has been associated with reduced subsequent disease activity and decreased need for active treatment (123, 124). Given the risks associated with immunosuppressive therapy, early intensive therapy or treatment of symptom-free patients can only be justified in those who are at high risk of a severe or disabling disease course. Clinical, biochemical, serological and genetic markers that are associated with a more severe disease course in patients with IBD have been identified but their individual and combined accuracy to predict disease course have yet been low (125, 126).

5 AIMS

5.1 GENERAL AIMS

The general aims of this thesis were to increase knowledge of the aetiology and prognosis of childhood-onset IBD.

5.2 SPECIFIC AIMS

5.2.1 Paper I

To describe the occurrence of paediatric IBD in northern Stockholm County during 2002-2007 as an extension of the previous study on the incidence of childhood onset IBD in northern Stockholm County covering 1990-2001.

5.2.2 Paper II

To test the hypothesis that antibiotic exposure during infancy and early childhood disrupts the homeostasis between host immune system and intestinal microbiota and thus increases the risk for CD later in life.

5.2.3 Paper III

To test the hypothesis that atypical microbial bowel colonisation, resulting from birth by caesarean section, leads to the development of a less stable homeostasis between host immune system and intestinal microbiota and thus increases the risk of CD during childhood.

5.2.4 Paper IV

To describe the prognosis in a population-based cohort of patients that have developed IBD during childhood.
To find predictors of a severe disease course in childhood-onset IBD and thus identify early in the disease course patients who may benefit from more intensive medical treatment.

6 MATERIALS AND METHODS

6.1 STUDY SUBJECTS AND METHODS

6.1.1 Paper I

All patients aged 0-15 years with paediatric IBD, diagnosed 2002-2007 and living in northern Stockholm County at time of diagnosis, were identified. Patients were identified by searching the records of patients at Astrid Lindgren Children's Hospital (which is the only paediatric gastroenterology unit in northern Stockholm County), the paediatric gastroenterology units in southern Stockholm County and in bordering counties, as well as all adult gastroenterology departments within the catchment area.

Patients were diagnosed according to the criteria that were used in earlier incidence studies in Stockholm and Sweden (71, 127-129):

To be given a diagnosis of IBD, the patient had to have a history of more than six weeks of diarrhoea, abdominal pain, weight loss, retarded growth or fever and an intestinal infection had to be ruled out by bacterial and parasitological tests.

1. A diagnosis of UC required:
    - Endoscopic findings of continuous mucosal inflammation involving the rectum and extending for variable distances in a proximal direction.
    - Histopathology in intestinal biopsies consistent with UC.

2. A diagnosis of CD required at least two of the following three criteria:
    - Endoscopic or radiographic findings of intestinal inflammation characterised by discontinuous intensity, ulcerations, cobblestoning or strictures.
    - Histopathology in intestinal biopsies consistent with CD.
    - Perinal or intestinal fistulas or abscesses.

3. A diagnosis of IBD unclassified (IBDU) was recorded if endoscopic, histological or radiographic evidence was compatible with inflammatory bowel disease but did not fulfil the criteria of UC or CD.

After revision, 133 children fulfilled the diagnostic criteria for IBD. Patients in the cohort were followed for a median of 5.8 years (range 1.0-8.9 years). If the initial IBD diagnosis changed during follow-up, the final diagnosis was used for calculations of incidence.

Data on the population in the catchment area (188 437 individuals <16 years of age lived in northern Stockholm County in 2005) during the study period were obtained from Statistics Sweden and the City of Stockholm.

6.1.2 Paper II

In the Swedish Inpatient Register we identified 1098 patients with CD born during the period 1973 to 1997. Each case was matched with up to six controls identified through the Swedish Medical Birth Register. Controls were selected at random among children born in the same delivery unit, in the same year and of the same sex. The matched sample and analysis was used to limit substantial confounding by temporal, geographic and social factors.

Seven common diagnoses that usually require antibiotic treatment were chosen as proxy markers of significant antibiotic exposure. Inpatient treatment for these diagnoses between birth and age five years for cases and controls was identified through the Swedish Inpatient Register.

The Swedish National Patient Register was started in the 1964 when the National Board of Health and Welfare started to collect information regarding inpatients at public hospitals. Initially the register only covered six counties in Sweden but over the next two decades the register expanded gradually. In 1984 the Ministry of Health and Welfare decided that participation was mandatory for all 21 counties in Sweden. From 1987 the National Patient Register thus includes information on all inpatient admissions to hospital and provides information on diagnoses and procedures for each stay. The quality of the inpatient register is checked regularly and it is estimated that over the last decade less than one percent of the records have been incomplete (130). Validation studies have shown that 85-95% of the diagnoses are accurately logged in the register (130). The use of the personal identity number (a ten-digit number maintained by the National Tax Board) given to all citizens in Sweden in the National Patient Register allows individual linkage to information contained in other national registers (131).

6.1.3 Paper III

Through the Swedish Inpatient Register, we identified 1536 patients diagnosed with paediatric (<16 years) CD between 1990 and 2006. Each case was matched with ten controls identified through the Medical Birth Register.

Information on birth mode and other markers of perinatal exposures, including maternal diagnoses during pregnancy, was provided by the Medical Birth Register. Information on neonatal infectious exposures for the child was provided by the Inpatient Register. Parental socioeconomic index was provided by the national Census that occurred nearest in time to the delivery.

The Swedish Medical Birth Register was founded in 1973 and includes data on almost all deliveries in Sweden. It is compulsory for every health care provider to report to the register and the information available is collected from medical records from prenatal, obstetric and neonatal care. Even though the basic structure of the register has remained unchanged, there have been some major modifications to content and methods of data collection over the years. For the main part of our study period it was possible to get full information from the Medical Birth Register on maternal diagnoses before and

during pregnancy and at delivery, maternal smoking during pregnancy, mode of delivery, birth weight and gestational age of the child and neonatal diagnoses.

6.1.4 Paper IV

Patient files for all the 280 patients diagnosed with paediatric IBD in the general population-based catchment area of northern Stockholm 1990-2007 were scrutinised from diagnosis until 2011. For patients diagnosed after 1997 most files were computerised and accessible using Stockholm County's patient file system. For patients diagnosed before 1997 the files had to be requested from the Stockholm County's patient file archives. For those patients who were referred to departments outside Stockholm during the follow-up period, copies of patient files were requested.

Of the 280 patients with IBD, 249 could be followed throughout the study period from diagnosis to 2011. In total, patients within the cohort were followed for 2727 person-years, giving a median follow-up time of 8.8 years (range 1.0-20.8 years). Due to loss to follow-up, 103 person-years could not be examined, corresponding to a loss of 3.7% of the maximum possible follow-up time.

By the end of the study period, all patients (100%) had been examined with colonoscopy (including visualization of the caecum). Of the 200 CD patients, 190 (95%) had been examined with ileoscopy, 188 (94%) with gastroscopy and 183 (92%) with small bowel imaging by the end of follow-up.

Patients were phenotyped within the framework of the Paris classification (40) **(Table 4)**.

Disabling symptoms were defined according to the criteria used in a study from the Saint-Antoine Hospital, Paris (126). The follow-up period for each patient was divided into five-year periods, with an assessment of disease burden in each of these periods. Patients were considered to have disabling symptoms if they had significant symptoms of diarrhoea, abdominal pain, fever, fatigue or EIM for more than one fifth of the time in each five-year period. Patients that underwent a colectomy or had a permanent stoma were defined as having continuous disabling symptoms.

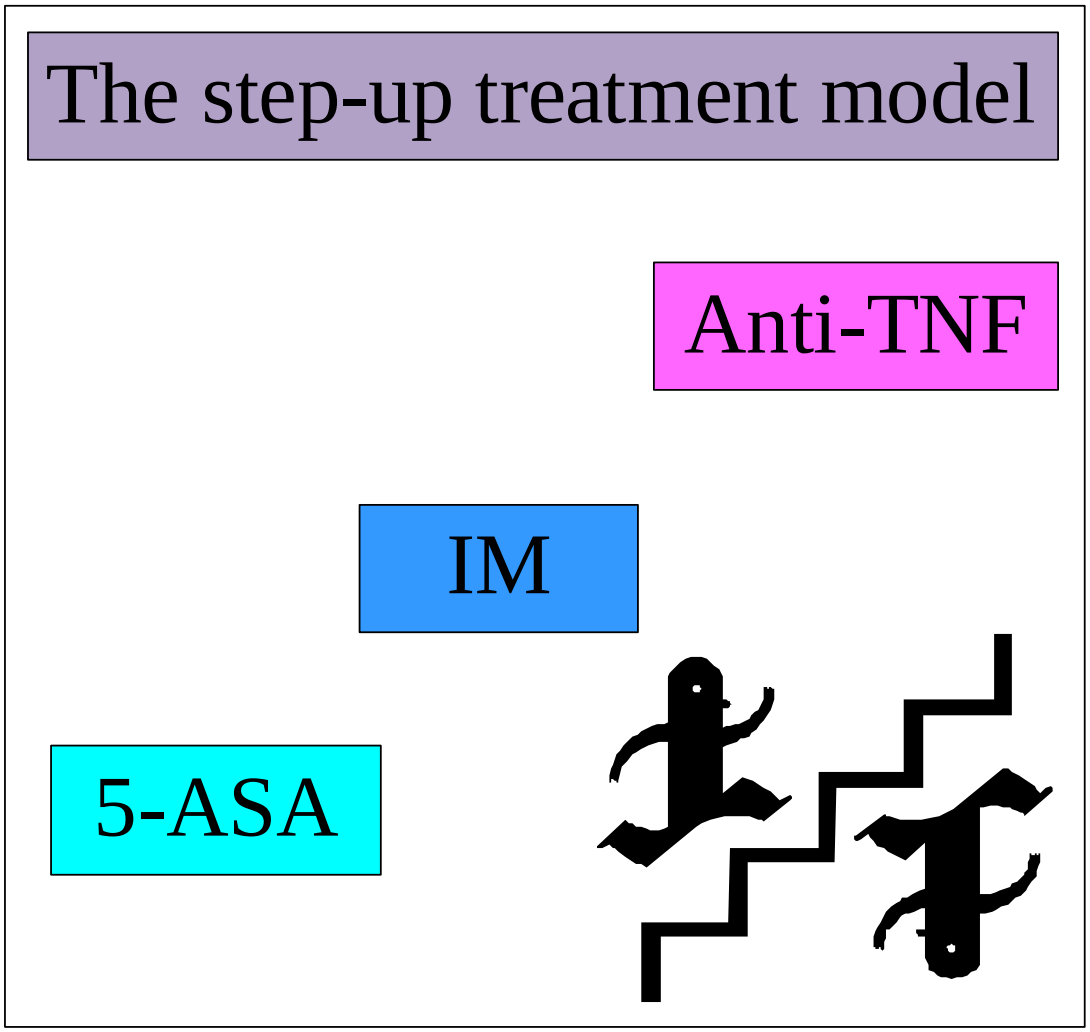
For each five-year period after diagnosis, patients were classified as corticosteroid-dependent if they were prescribed more than two steroid courses or required more than three months continuous treatment (126).

Medication at 5, 10 and 15 years after diagnosis was categorised according to the step-up model (**Figure 1**).

Disease activity as shown by routine follow-up endoscopy at one year was defined by the endoscopist as mucosal healing or persistent erosive mucosal inflammation.

A complicated disease course in patients with CD was defined as progression to extended location or complicated behaviour or need for intra-abdominal surgery during follow-up.

Figure 1
Maintenance therapy categorised according to the step-up model in which 5-ASA represents step one, IM (azathioprin, mercaptopurin and methotrexate) step two and anti-TNF (infliximab and adalimumab) step three.



6.2 STATISTICAL ANALYSIS

6.2.1 Paper I

Yearly incidence rates were standardized to the Swedish population in 2002 by sex and age. Confidence intervals for incidence rates were calculated assuming a Poisson distribution. Trends in incidence rates were calculated using a Poisson regression model.

6.2.2 Paper II

Conditional logistic regression was used to analyse associations of inpatient treatment for diagnoses associated with antibiotic exposure, with risk for CD. The association of each diagnosis with CD risk was tested in univariate analysis. Where statistically significant associations were observed these diagnoses were included in a multivariate logistic regression analysis. In the final model the association of inpatient treatment for pneumonia with CD risk was also adjusted for markers of exposure that in former studies have been associated with CD risk and so may constitute potential confounding factors.

6.2.3 Paper III

Conditional logistic regression was used to analyze associations of caesarean section with paediatric CD risk. The analysis was adjusted for established risk factors for CD and for exposures during pregnancy and after delivery in the study that were associated with an increased risk for paediatric CD.

6.2.4 Paper IV

The cumulative proportion of patients that progressed to complicated disease behaviour or underwent intra-abdominal surgery or immunosuppressive therapy was estimated by the Kaplan-Meier method. Differences in survival between those diagnosed in the early or in the late part of the study period were assessed using the Log rank test.

Cox proportionate hazard regression was used to analyze the association of patient characteristics at diagnosis and during early follow-up with a complicated disease course. The association of early patient characteristics with a complicated disease course was tested in univariate and multivariate analyses.

In an additional analysis not included in the paper, the performance of the selected variables in the Cox model to predict requirement for intra-abdominal surgery between two and ten years after diagnosis was tested using logistic regression. The sensitivity, specificity, positive and negative predictive values of these measures were calculated.

6.2.5 Overall

In all papers (I-IV) normally distributed continuous variables are presented as mean and standard deviation. Measures with a skewed continuous distribution are presented as

median and range. Categorical variables are presented as proportions and compared using the chi-square test or Fishers exact test for small sample sizes.
In all papers the statistical significance level was set at $p < 0.05$.

Analyses in paper I and IV used SPSS. Analyses in paper II-III used the SAS software package.

6.3 ETHICS

Approval for the studies reported in all papers was obtained from the Regional Ethical Review Board at Karolinska Institutet, Stockholm.

For **paper I**; Dnr 04-915/1, Dnr 2009/1435-31/3
For **paper II;** Dnr 2005/1232-31, Dnr 2011/1352 32
For **paper III**; Dnr 2005/1232-31, Dnr 2006/1043-32, Dnr 2008/314-32
For **paper IV**; Dnr 20011/120-31, Dnr 2012/1125-32

7 RESULTS

7.1 PAPER I

During the study period (2002-2007), 133 patients were diagnosed with paediatric IBD (96 (72%) with CD, 29 (22%) with UC and 8 (6%) with IBDU) in northern Stockholm County.

The standardised incidence (per 105 person-years) of IBD was 12.8 (95% confidence interval (CI) 10.8-15.2), 9.2 (95% CI 7.5-11.2) for CD and 2.8 (95% CI 1.9-4.0) for UC.

An increasing incidence rate of UC (58.4% (CI 22.8%-104.3%, $p<0.01$)) was observed during the study period. No statistically significant temporal trend was observed for the incidence of IBD (3.2% (95% CI -6.6%-14.0%, $p=0.54$)) or CD (-8.8% (95% CI -18.6%-3.0%, $p=0.14$)) during the study period.

A statistically significant male predominance was observed for CD (sex ratio 1.5 (95% CI 1.0-2.3, $p<0.05$)).

The incidence rate of IBD in northern Stockholm County during this study period (2002-2007) was significantly higher than during the previous six-year period 1996-2001 (4.8% (95% CI 0.3%-9.5%, $p<0.05$)). When the entire study period of 1990-2007 was analysed, we found significant temporal trends for increasing incidence rates of overall IBD (6.6% (95% CI 4.1%-9.2%, $p<0.01$)) and CD (7.7% (95% CI 4.7%-10.8%, $p<0.01$)). No significant temporal trend in incidence rate of UC (3.3% (95% CI -1.4%-8.1%, $p=0.17$)) could be detected for the entire study period of 1990-2007.
(See **Figure 1**, paper III, page 33)

7.2 PAPER II

Inpatient treatment for pneumonia before five years of age was the only diagnosis that was significantly associated with subsequent CD. Inpatient treatment for otitis media was positively associated with CD although the association did not reach statistical significance.

When pneumonia and otitis media were included in the same conditional regression model, the association of otitis media with CD was attenuated, while the association of pneumonia with CD remained significant.

The adjusted associations (adjusted also for maternal age) of inpatient treatment for pneumonia between birth and age five years were significant with both childhood-onset CD (odds ratio (OR) 2.74, 95% CI 1.04-7.21) and adult-onset CD (OR 4.94, 95% CI 1.83-13.23).

7.3 PAPER III

7.3.1 Overall caesarean section

Birth by ccaesarean section was not associated with an increased risk of paediatric CD. However, examination of males and females separately revealed a modestly increased risk for paediatric CD among boys delivered by caesarean section. The association of caesarean section with paediatric CD among boys was statistically significant even after adjustment (adjusted for maternal IBD, maternal age, number of older siblings and gestational age) (OR 1.25, 95% CI 1.01-1.54).

7.3.2 Elective caesarean section

In infants born at term after 1981 we could analyse the association of caesarean section, divided into elective and acute, with paediatric CD. In this subset elective caesarean section was associated with a statistically significant raised risk of paediatric CD. Maternal urinary tract infection was found to be significantly associated with risk for paediatric CD in this subset and was therefore introduced as a potential confounder. Adjustment for potential confounding factors, including maternal urinary tract infection, did not eliminate the statistical significance of the association (OR 1.36, 95% CI 1.02-1.80).

7.4 PAPER IV

7.4.1 Childhood-onset IBD

During the study period (1990-2007) 280 patients were diagnosed with childhood-onset IBD (200 (71%) with CD, 74 (26%) with UC and 6 (2%) with IBDU) in northern Stockholm County.

The proportion of IBD patients that were classified as having disabling symptoms decreased significantly over time (1-5 years: 32% and 11-15 years: 15%, $p<0.01$).
(See **Figure 1**, paper IV, page 22)

The proportion of IBD patients that did not use any prescribed medication significantly increased over time (at 5 years: 6% and at 15 years: 28%, $p<0.01$).
(See **Figure 2**, paper IV, page 23)

The proportion of IBD patients that were classified as corticosteroid dependent significantly decreased over time (1-5 years: 31% and 11-15 years: 14%, $p<0.01$).

7.4.2 Childhood-onset UC

Pancolitis (E4) was the most common disease presentation in patients with UC.

Extended intestinal inflammation during follow-up was observed in 6 (22%) of the 27 UC patients that did not present with pancolitis (E4).
(See **Figure 3**, paper IV, page 24)

The cumulative risk for colectomy in patients with UC, five and ten years after diagnosis, was 6% (95% CI 2%-12%) and 8% (95% CI 4%-20%) respectively.

7.4.3 Childhood-onset CD

In patients with CD, isolated colonic disease (L2) was the most common disease location. Twenty-nine patients (14%) with CD disease had a complicated disease behaviour at diagnosis (10 (5%) presented with intestinal complications (B2, B3) and 19 (10%) with perianal fistulas or deep fissures (+P)).

Extended disease location over time was observed in 29 (15%) of the 189 CD patients that did not present with pan-enteric disease (L3+L4). By the end of follow-up, pan-enteric disease had been observed in 21 (11%) of the 200 CD patients.
(See **Figure 4**, paper IV, page 24)

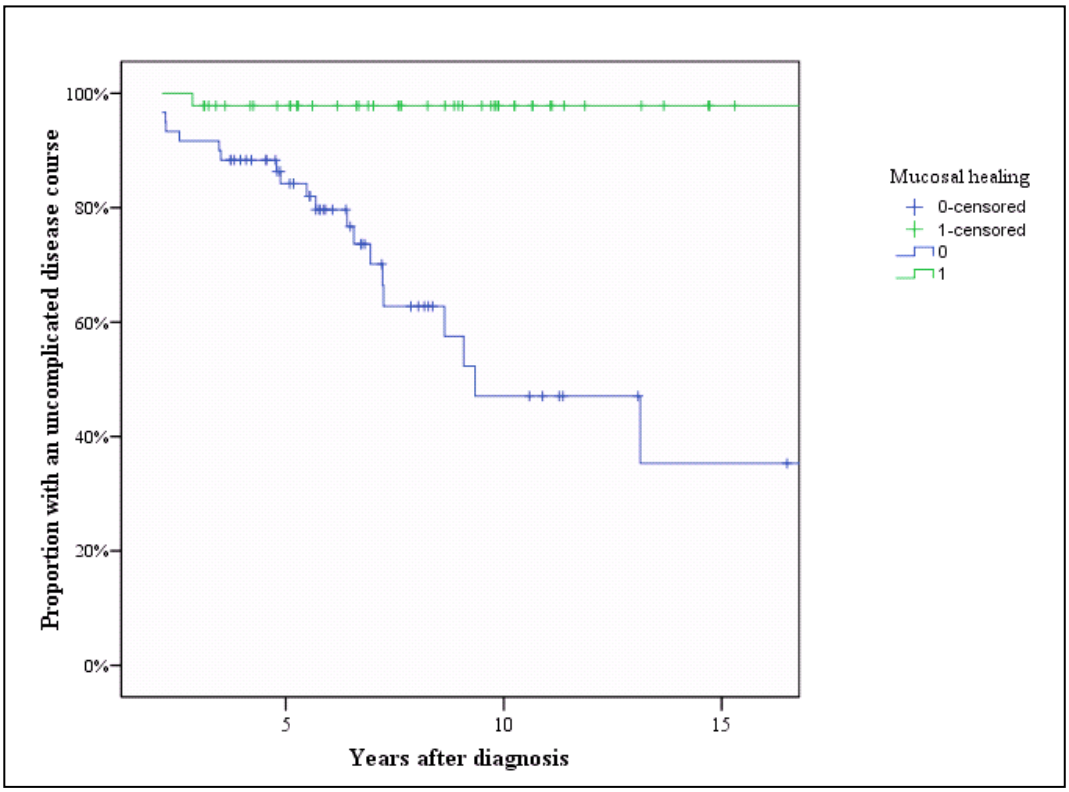
Among the 190 CD patients that presented with non-stricturing, non-penetrating intestinal inflammation, 25 (13%) developed complicated disease behaviour during follow-up. (See **Figure 5**, paper IV, page 25)
By the end of the study period perianal fistulas had formed in 17 (9%) of the 181 CD patients that were not afflicted with perianal disease at diagnosis.

The cumulative risk for intra-abdominal surgery in patients with CD five and ten years after diagnosis was 13% (95% CI 8%-18%) and 22% (95% CI 15%-28%) respectively.

In the multivariate Cox proportionate hazard regression analysis, persistent erosive mucosal inflammation at one year re-endoscopy was the only characteristic that was significantly associated with an increased risk (hazard ratio 14.56 (95% CI 1,79–118,68) of a complicated disease course (defined as progression to extended location or complicated behaviour or need for intra-abdominal surgery) two years after diagnosis. (**Figure 2**)

In the sensitivity and specificity analysis, we tested the predictive performance of the only selected variable in the multivariate Cox regression model. More specifically, we tested the ability of persistent erosive mucosal inflammation at one year re-endoscopy to predict progression to a complicated disease course in a subset of 87 patients that presented with uncomplicated disease and who were re-endoscoped between six months and two years after diagnosis and had more than five years of follow-up. Persistent mucosal inflammation at one year re-endoscopy had a sensitivity of 95%, a specificity of 56%, a positive predictive value of 60% and a negative predictive value of 97% for a complicated disease course between two and ten years after diagnosis within the sub-cohort.

Figure 2 shows a Kaplan-Meier curve on the progression rate to a complicated disease course two years after diagnosis by mucosal healing at one year re-endoscopy for patients (n=106) presenting with an uncomplicated childhood-onset CD in northern Stockholm 1990-2007.



8 DISCUSSION

8.1 STRENGHTS

The strengths of this thesis include that we present data on incidence (**paper I**) and prognosis (**paper IV**) of childhood-onset IBD from a relatively large cohort based on the general population, with patient information collected continuously over 18 years, and that nearly all the patients could be followed, giving a median follow-up time of almost 10 years after diagnosis.

Another major strength of this thesis is that we could use the prospectively collected data in Swedish national population registers and thus identify a large number of patients with childhood-onset CD. This allowed us to study the possibly weak (but from an aetiological perspective important) associations of early life exposures with risk of CD and adjust the analysis for several important potentially confounding factors (**paper II and III**).

8.2 WEAKNESSES

The major weakness of the two regional general population-based cohort studies in this thesis (**paper I and IV**) is the relatively small number of patients; this limits the possibility of detecting small changes in incidence over time and confirming observed differences in disease presentation and prognosis.

The major weakness of the two national register studies in this thesis is that we could only adjust for factors that were collected in the national registries; thus the associations we demonstrated might have been confounded by unreported yet important exposures such as diet, smoking and drug exposure (**paper II and III**).

8.3 INCIDENCE

The observed increasing incidence of paediatric IBD and the predominance of CD in northern Stockholm County in this thesis (**paper I**) are consistent with global trends. During recent decades increasing incidence rates of both childhood-onset UC and childhood-onset CD have been reported in epidemiological studies from most continents (69). However, it is worth noting that the increase in the incidence of IBD had stagnated and that the incidence of CD had even decreased at the end of the study period. The plateauing incidence of paediatric IBD in our study contrasts with most contemporary incidence reports. Future studies will establish whether we have observed a permanent shift from low to stable high incidence rates, a transition pattern that was observed in adult-onset IBD incidence studies from North America and Western Europe during the 1950s-1970s (65).

Increasing incidence of immune mediated diseases (including allergic and autoimmune diseases and IBD) has been observed in Europe and North America during the last decades. Although the rise in some of these diseases might be attributed to an increased awareness and improved diagnostic procedures, it is worth noting that the incidence of

childhood-onset Diabetes Mellitus type 1 (DM1) has increased dramatically in Scandinavia during recent decades (132, 133). DM1 has a rather precise definition and is fatal when untreated, so it is implausible that the increase in incidence could be explained by changes in diagnostic procedures or improved awareness of the disease. In northern Stockholm the increase in paediatric IBD incidence at the end of the 1990s was paralleled by a steep increase in childhood-onset DM1 incidence (71, 134). It should also be noted that the faecal calprotectin test as a screening tool for children with long-standing gastrointestinal problems was introduced in northern Stockholm after the transition to high incidence rates of paediatric IBD. It thus seems more plausible that the observed increase in childhood-onset IBD rates is real and reflects a less stable gastrointestinal immunological balance in at least some children born during the last three decades.

The higher incidence rate of CD than UC observed in our study is consistent with most epidemiological studies on paediatric IBD from the last decade. However, an inverse distribution of the two diseases was recently reported from the Uppsala region, just north of Stockholm, in a contemporary childhood-onset IBD cohort (63, 135). Striking differences in CD/UC ratio between geographically, socioeconomically and genetically comparable countries have also been reported, implying that these might rather reflect differences in classification than true discrepancies. Misclassification could be expected to be more common within childhood-onset IBD, as colitis is the most common disease presentation in children with CD and thus makes differentiation from UC more complicated. The creation of more specific common diagnostic criteria for paediatric IBD would allow international comparisons to be made with greater certainty (136).

Reported incidence rates of paediatric IBD from the Scandinavian countries are among the highest in the world. However, the rates seem to differ significantly between the neighbouring countries (72, 137, 138). The differences in IBD incidence could hardly be explained by differences in health care provision or general awareness of IBD, as all the Scandinavian countries have similar health care organisation and share a long-standing interest in IBD incidence studies. This finding should prompt further comparative studies in search of environmental factors that have changed recently and could explain the ongoing global, but by country borders varying, epidemic of paediatric IBD (65).

8.4 PNEUMONIA AND CAESAREAN SECTION

In papers **II and III** in this thesis we made use of Swedish national population registers to conduct hypothesis testing case-control studies. We demonstrated that inpatient treatment for pneumonia during early childhood and birth by elective caesarean section were associated with significantly increased risks for CD. These findings lend some support to the hypothesis that atypical first or early disrupted microbial colonisation of the bowel might hamper the development of stable homeostasis between immune system and bowel flora and thus increase the risk of CD later in life in susceptible individuals.

In **paper II** we used inpatient treatment as a proxy marker of significant antibiotic treatment as by that time no Swedish register for drug prescription was available for studies such as ours. As most antibiotic treatments for children are prescribed in outpatient settings (139) the extent of the difference in true exposure to antibiotics between cases and controls might be questioned. Recently published studies from Denmark and Finland that have used nationwide databases of antibiotic purchases have confirmed the association of antibiotic prescription during early childhood with an increased risk for CD later in childhood (140) (141). The association of early prescription of antibiotics with risk of CD in adulthood (where we found the strongest association) was not studied in these papers. As the influence of genetic traits could be expected to be stronger for patients that develop CD early in life it is possible that adult- rather than childhood-onset CD is of even greater interest when evaluating the influence of early environmental exposures. Although the association of early antibiotic use with CD risk seems consistent it should be emphasised that the association might be explained by immune dysregulation causing increased susceptibility to both early bacterial infections and later CD in some individuals. However, the absence of an association of inpatient treatment for pneumonia after five years of age with CD risk later in life in makes this explanation less plausible.

By using national registers in **paper III** we identified a large number of patients and thus were able to examine a possibly low-magnitude, but aetiologically interesting, association, such as birth mode, with CD risk. By focusing on patients with childhood-onset CD we were able to adjust for some potentially important confounding maternal factors (such as urinary tract infections and smoking during pregnancy) that had been added more recently to the National Medical Birth register. Another reason why we restricted the analysis to childhood-onset disease is that children have not yet been exposed to environmental factors to the same degree as adults, so the association of birth mode with paediatric CD is less likely to be confounded by unmeasured exposures such as smoking. We demonstrated that birth by elective caesarean section was associated with a significantly increased risk for CD. The association of overall caesarean section with increased risk of CD among males (who constitute the main proportion of paediatric CD patients) gave further strength to our interpretation that birth mode may influence CD risk during childhood. Our conclusion is also supported by a recent national cohort study from Denmark that found that individuals born by caesarean section were at a modest but significantly increased risk of developing IBD, both CD and UC, during childhood (142). However, the Danish findings do somewhat question our interpretation as the authors found a more pronounced risk among children delivered by acute caesarean section. These findings suggest that the increased risk for IBD seen among children delivered by caesarean section might not be explained by an atypical first bacterial colonisation, but by the factors that prompted non-elective caesarean section. Breast feeding has been reported to be less common among infants delivered by caesarean section (143) seems to reduce the risk of IBD (86). Neither of these two national register studies allowed adjustment for early feeding pattern and it is possible that early breast feeding might be a causal factor. Although the apparently consistent association of birth mode with paediatric CD is of aetiological interest, the association is too modest to suggest that advice on delivery procedures should be altered. Although the proportion of children that are delivered by caesarean section has

increased more than threefold during the last decades in Sweden (144) this change in delivery practice may at the most (assuming a causal association) explain risk in a very small fraction of the 100 or so children (135, 145) who are now diagnosed annually with CD in the Sweden.

It could be speculated that the increase in prescription of antibiotics to children in Sweden, which lasted until the mid-1990s (139) could have contributed to the increasing incidence rates of childhood-onset CD during the past two decades (63, 71, 128, 129, 135, 145). If this is true one would expect to see a decline in paediatric CD incidence rate in the next decade, as the prescription of antibiotics to children 0-4 years of age fell by 37% between 1995 and 2004 in Sweden (139). Results from a comparative European study covering 1994-1997 argue against early use of antibiotics being of major importance for the epidemic of childhood-onset CD in Scandinavia, as prescription of antibiotics in a country such as Spain, with low incidence of paediatric CD (146) was estimated to be almost twice as high as in Sweden (147).

8.5 PRESENTATION AND PROGNOSIS

In **paper IV** we confirmed that the childhood-onset IBD phenotype, compared to the adult-onset phenotype, seems to be characterised by more extensive disease at diagnosis. However, the predominance of isolated colonic disease observed in our CD cohort conflicts with reports from most other contemporary paediatric IBD cohorts (114, 116, 148). These suggest a paediatric CD phenotype characterised by even more widespread intestinal inflammation, often involving the large and small bowel as well as the upper gastrointestinal tract (pan-enteric disease). The significantly different disease distribution observed in our cohort might reflect real differences. However, the distinct difference in dominating phenotype – a difference seen even between the Scandinavian countries (148) – rather suggests differences in classification. This speculative explanation emphasises the importance of implementing the recently created Paris paediatric IBD classification (40) as a common platform for upcoming paediatric studies.

The large majority of the patients in this relatively large population-based childhood-onset IBD cohort could be followed until the end of the study period. Follow-up over a median of almost ten years did not lend any support to the hypothesis that childhood-onset IBD represents a particularly dynamic and aggressive IBD phenotype (114, 116, 117, 149). The complication and intra-abdominal surgery rates were significantly lower in our cohort than those observed in earlier studies of population-based childhood-onset IBD cohorts (116, 117, 150, 151). The patients in our cohort had, compared with contemporary adult-onset IBD cohorts, similar stability of disease location over time and comparable or somewhat lower complication and surgery rates (21, 25, 152). Our findings are consistent with recently published comparative studies from Hungary and Canada that could not find differences in progression to complicated disease behaviour or requirement for surgery between childhood- and adult-onset CD cohorts (150, 153). Prospective studies that compare presentation and progression of IBD between cohorts

of children and adults with a common standardized diagnostic and follow-up protocol would be of great interest to further explore the differences in phenotype by age.

The observed relatively mild and decreasing disease burden in our cohort over time implies that the natural disease course in childhood-onset IBD might be less aggressive than previously believed. This finding is major importance as the focus of IBD treatment is now shifting from symptom control to prevention of irreparable intestinal damage (154). This treatment strategy has attracted great interest among paediatric gastroenterologists, as childhood-onset IBD has long been considered a more aggressive phenotype and as paediatric patients have to face a lifetime accompanied by disease. The ongoing era of biologics has raised hopes that modern immunosuppressive treatments should have the capacity to change the natural disease course of IBD (108). Most biologics have initially been tested in rheumatological diseases and then later moved on to trials for other inflammatory diseases such as IBD. The frontline experience gained in treating rheumatoid arthritis has thus served as an inspiration for gastroenterologists when trying to develop care for patients with IBD (154). The concept of treating even subclinical inflammation in rheumatoid arthritis is now well established, as treatment guided by biochemical inflammatory markers has been associated with reduced joint destruction and lower levels of functional disability (155-157). Analogous with this concept, earlier and more intense pharmacological treatment to prevent structural damage to the intestine is now also advocated by some experts in IBD (154). Most attention has been directed to CD, where there is a weaker association between symptoms and intestinal inflammation (122) and complications (strictures and fistulas) requiring intra-abdominal surgery seem to develop in almost all patients over time (27). To alter the natural disease course of CD it is suggested that treatment choices should be guided not only by symptoms but also by more objective markers of intestinal inflammation (158). Intestinal mucosal healing has repeatedly (as demonstrated in our cohort) been associated with a low risk of developing intestinal complications in prognostic studies (123, 159). Some experts thus recommend that treatment of CD should aim to achieve and maintain both clinical remission and mucosal healing (a combination for which the term deep mucosal healing is proposed) (160). A randomised controlled trial (REACT-2) was started in 2013 investigating whether an accelerated step-up treatment guided by deep remission leads to fewer complications as compared with the classical symptom-guided step-up approach. In this first trial aimed at modifying the course of CD, the recently developed intestinal damage scores will be used as endpoints, in addition to complication and surgery rates (161). This and hopefully other forthcoming trials will provide evidence of the capacity of IM and anti-TNF to prevent structural damage to the intestine and thus their potential to modify the natural disease course in IBD.

The concept of treating beyond symptom control might be less controversial in paediatric CD, where impaired growth is already used as a marker of significant inflammatory activity that prompts more active treatment, even in the absence of overt gastrointestinal symptoms. However, if one looks to the rheumatology experience for guidance, it should be kept in mind that treatment concepts designed for joint inflammation probably have to be adapted to suit the gastrointestinal tract. Even if immunosuppressive therapy turns out to have the potential to prevent intestinal fibrosis,

this might not be a concern from the patients' perspective. Intestinal damage may not necessarily cause a functional disability in the way a destroyed joint will (162). As demonstrated in our study, several of the patients with CD who had significant fibrotic tissue transformation (strictures) also had relatively mild symptoms during the following years and did not require intra-abdominal surgery. Future modifying studies of IBD should thus ideally also include the newly developed functional disability index for IBD patients as endpoint (163). When comparing with current treatment concepts in rheumatology it should also be noticed that disease-modifying treatment in this disease does not seem to be associated with an increased risk for development of lymphoma (164). On the contrary immunosuppressive treatment in CD entails a modest but yet increased risk for development of fatal lymphoma in young males with IBD (165). Given the risks associated with immunosuppression in IBD, early intensive therapy could only be justified in those patients who face a high risk of a complicated disease course. The risk that a patient with rheumatoid arthritis will develop a disabling disease course can to some extent be predicted at diagnosis by presence of auto-antibodies, levels of biochemical inflammatory markers and joint erosion scores (166). In contrast prognostic studies in CD have so far demonstrated low performance of combined early clinical, endoscopic, biochemical, serological and genetic markers to predict a complicated disease course (125, 167). The relatively mild disease course of the patients in our cohorts suggests that a substantial proportion of childhood-onset CD patients might not need disease modifying treatment and that aiming for symptomatic alleviation in these patients might be sufficient. For the time being, the initiation of treatment aiming beyond symptomatic control might only be justified in paediatric CD patients that present with early significant intestinal (or perianal) damage (deep ulcerations or stricturing or fissuring disease behaviour (25, 116, 151, 168)). The stronger association of non mucosal healing than persistent symptoms with development of a complicated disease course in our study suggests that maintenance treatment intensity in this high risk group should be guided by disease activity assessments by endoscopy.

9 CONCLUSIONS

- The incidence of paediatric IBD in northern Stockholm 2001-2007 was higher than the rates observed 1990-2001. The previous rapid increase in CD incidence seems though to have levelled out. The incidence rate of paediatric IBD in northern Stockholm was consistently higher than those reported from most other regions in the world.

- The association between CD and both birth by elective caesarean section and inpatient treatment for pneumonia prior to the age of five years gives some credence to the theory that atypical or disrupted bowel colonisation during early childhood might increase the risk for CD later in life.

- Patients with childhood-onset IBD were characterised by extensive colitis that was relatively stable over time and associated with a relatively low risk of complications and intra-abdominal surgery. Our findings confirm the more extensive disease location in paediatric IBD but question the proposed dynamic and aggressive nature of the childhood-onset IBD phenotype that has been indicated previously.

- The strong association of mucosal healing at one year with an uncomplicated disease course suggests that endoscopy should be used to guide treatment intensity in childhood-onset CD.

10 CHALLENGES

Although knowledge concerning paediatric IBD is rapidly growing much research is yet to be done.

The global epidemic of childhood-onset IBD seem to be explained by changing environmental exposures associated with the modern lifestyle that is now enjoyed by most people in high and middle income countries. The observed increase in IBD incidence in Stockholm does not seem to be explained by increasing use of either antibiotics or caesarean section. However the association of these exposures with CD risk suggests that an atypical (from an evolutionary perspective) pattern of bowel colonisation early in life might alter the susceptibility to disease later life in some individuals.

The recent mapping of the full human microbiome has gained much attention and fuelled interest in the intricate symbiosis or commensalism between host and microbiota and its implications for human health (169). Future research ought to explore further the role of the bowel microbiota in the pathogenesis of IBD; to characterise the pattern of bowel flora before disease onset in those who later in life develop IBD (170); to examine whether modification of the bowel flora with pre- or probiotics might have an impact on disease activity in already afflicted patients (171); to study whether transplantation of faecal matter from a healthy donor is a way of correcting the imbalance the between immune system and the bowel microbiota in patients with IBD (172); to study if there is an association between IBD and modern agriculture's increasing use of biocides that are potentially harmful to bowel flora (173).

The incidence trends in paediatric IBD should be studied consistently in high, middle and low income countries. Assuming that antibiotic treatment and birth mode (and other factors associated with these, possibly with a greater effect on bowel colonisation) have some impact on IBD incidence, one might expect a steep increase in paediatric IBD incidence rates, during the next decades, in the rapidly developing low and middle income countries of Eastern Asia, where most children now seem to have a high exposure to antibiotics and the rate of caesarean section is increasing (174, 175).

The concept of treating of paediatric IBD with immunosuppression has been questioned as onset of IBD in early childhood seems to be associated with defective anti-inflammatory responses in some patients (55). Nevertheless this concept could be the ruling paradigm for some years to come as several studies have proven that immunosuppressive therapy effectively relieves symptoms and promotes growth in paediatric IBD patients (176-179). New biologics with somewhat different modes of action than anti-TNF are now on the verge of being added to the pharmacological repertoire for the treatment of paediatric IBD (180-182). The rare but sometimes severe side effects of immunosuppressive therapy (165, 183) suggest that these drugs should be reserved for patients that have disabling symptoms or face a substantial risk of developing complications. Future studies ought to further study the association of

clinical, endoscopical, histological, radiological, biochemical, genetic, serological and microbial characteristics with severe disease course in order to build predictive models that can identify high risk groups as early as at diagnosis, groups for whom the advantages of immunosuppression could perhaps outweigh the risks (184). Taking the unique features of paediatric IBD into consideration, the performance of these models should also be tested in population-based cohorts of patients with childhood-onset IBD. Future studies should also address whether non-invasive measures of disease activity such as faecal calprotectin or imaging techniques could be used to guide the treatment intensity in IBD patients (122, 185, 186). The hypothesis that early immunosuppressive therapy may have the potential to prevent the transition from acute to chronic inflammation and thus alter disease progression to follow a more benign course should be elaborated further (187).

11 SAMMANFATTNING PÅ SVENSKA

Crohns sjukdom (CD) orsakas av en kronisk inflammation som kan uppträda i hela mag-tarmkanalen. Vid CD kan tarminflammationen leda till bildning av sårgångar genom tarmväggen (fistlar) och förträngningar av tarmen på grund av ärromvandling (strikturer).
Ulcerös kolit (UC) orsakas av en kronisk inflammation som alltid uppträder i ändtarmen men som även kan omfatta hela tjocktarmens slemhinna.
CD och UC uppträder ofta med samma symptom och liknande inflammationsutbredning och kräver ofta likartade behandlingar varför de båda sjukdomarna inte sällan med ett gemensamt namn kallas för inflammatoriska tarmsjukdomar (IBD).

Orsaken till varför IBD uppkommer är fortfarande ofullständigt känd. Studier av tvillingar har visat att ärftliga faktorer spelar stor roll för uppkomsten av IBD. Under de senaste två decennierna har antalet barn och ungdomar som insjuknar i IBD i världen ökat. Den ökande incidensen av IBD bland barn och ungdomar kan inte förklaras med förändringar i arvsanlagen utan måste förklaras med ändrad livsmiljö. IBD antas kunna förklaras med en förlust av den tidigt i livet förvärvade immunologiska toleransen gentemot tarmens bakterieflora. Allmänt förbättrade levnadsvillkor under de senaste decennierna har medfört att barn idag konfronteras med färre och delvis andra mikroorganismer än tidigare generationer. Hos barn som växer upp i rika länder kolonialiseras tjocktarmen efter födelsen av delvis andra bakterier än de som tidigare generationer anpassat sig till. Det är möjligt att denna ur ett evolutionärt perspektiv avvikande kolonisation skapar en mindre stabil jämvikt mellan tarmfloran och immunförsvaret. En sådan obalans skulle kunna leda till en ökad risk för att insjukna i IBD senare i livet. Flera arbeten som publicerats under de senaste åren har visat att de som insjuknar under barn- och ungdomsåren i IBD verkar har en sämre prognos än de som insjuknar i vuxen ålder. Man har därför antagit att IBD som debuterar under barnaåren utgör en svårare form av sjukdomen med ökad risk för komplikationer (strikturer och fistlar) vilka ofta kräver kirurgisk behandling. Nya undersökningar studier talar för att tidigt insatt behandling med immunförsvarsdämpande läkemedel har minskat behovet av bukoperationer. Behandling med immunförsvarsdämpande läkemedel kan dock öka risken för att drabbas av ovanliga men allvarliga infektioner och hos pojkar och unga män även lymfkörtelcancer. Behandling med immunförsvarsdämpande läkemedel kan därför bara försvaras hos de barn och ungdomar som löper en stor risk för att drabbas av ett allvarligt sjukdomsförlopp.

Syftet med denna avhandling var att följa utvecklingen av incidensen av IBD bland barn och ungdomar, att testa om en avvikande eller tidigt störd bakteriell kolonisation av tarmen var kopplad till en ökad risk för att drabbas av CD senare i livet, att beskriva prognosen vid insjuknande i IBD under barn- och ungdomsåren och att testa om tarminflammationens karaktär tidigt i sjukdomsförloppet kan användas för att förutse vilka patienter som löper en ökad risk för att drabbas av ett mer allvarligt sjukdomsförlopp.

I artikel I genomförde vi en uppföljande studie av hur incidensen av IBD bland barn och ungdomar i norra Stockholms län utvecklats mellan 2002 och 2007. Efter journalgenomgång kunde vi konstatera att 133 barn och ungdomar insjuknat i IBD under tidsperioden vilket motsvarar en incidens (per 100 000 barn och år) om 12,8 (95% konfidens intervall (CI) 10,8–15,2) för IBD, 9,2 (95% CI 7,5–11,2) för CD och 2,8 (95% CI 1,9–4,0) för UC. Incidensen av pediatrisk IBD under tidsperioden var signifikant högre än i vår tidigare studie som omfattade åren 1990-2001. Under studieperioden noterades en statistiskt signifikant ökning av incidensen i UC. Den stadiga ökningen av incidensen av CD som vi observerade under den förra studieperioden verkade dock ha avstannat även om incidensen sett ur ett globalt perspektiv fortfarande var mycket hög.

I artikel II studerade vi om sjukhusvård för diagnoser som kräver antibiotikabehandling ökade risken för att drabbas av CD senare i livet. Med hjälp av svenska befolkningsregister kunde vi identifiera 1 098 patienter födda mellan 1973 -1997 med CD och 6550 matchade kontroller. Vi kunde konstatera att sjukhusvård före fem års ålder (men inte senare) för lunginflammation var kopplat med en ökad risk för att insjukna i CD både under barn- och ungdomsåren (odds kvot (OR) 2,74, 95% CI 1,04–7,21) och i vuxen ålder (OR 4,94, 95% KI 1,83–13,23). Slutsatsen var att lunginflammation, och därigenom antiobiotika-behandling, tidigt i livet var förenat med en ökad risk för att senare i livet drabbas av CD och att detta samband antingen förklaras av gemensam sårbarhet för de båda sjukdomarna eller avspeglar ett orsak-verkans-samband.

I artikel III undersökte vi om förlossningssätt påverkade risken för att insjukna i CD under barn- och ungdomsåren. Vi identifierade 1 536 patienter som mellan 1990 och 2006 fått diagnosen IBD under barn och ungdomsåren och matchade dem med 15 439 kontroller via svenska befolkningsregister. Uppgifter om omständigheterna före, under och efter förlossningen hittade vi i det nationella medicinska födelseregistret. Förlossning med kejsarsnitt var kopplat med en något ökad risk för att insjukna i CD under barn- och ungdomsåren för pojkar (OR 1,25, 95 % CI 1,01–1,54) men inte för flickor (OR 0,99, 95% CI 0,76–1,29). En något ökad risk för CD bland både flickor och pojkar sågs bland de som förlösts med planerat kejsarsnitt (OR 1,36, 95% CI 1,02–1,80). Slutsatsen var att kejsarsnittsförlossning (eller andra med förlossningssättet sammanhängande faktorer) något ökade risken för att insjukna i CD under barn och ungdomsåren och då framförallt bland pojkar .

I artikel IV beskrev vi prognosen för de alla de 280 patienter som insjuknade i IBD under barn- och ungdomsåren i norra Stockholms län mellan 1990 och 2007 under en medianuppföljningstid om 8,8 år med ledning av journaldata. I denna populationsbaserade kohort kunde vi visa att andelen patienter med IBD som upplevde handikappande symptom minskade statistiskt signifikant under uppföljningstiden. Vid insjuknandet konstaterades att flertalet av patienterna med både UC och CD hade en utbredd men till tjocktarmen begränsad tarminflammation. Tio år efter diagnos hade 8% (95% CI 4%-20%) av UC patienterna behövt genomgå kolektomi (operativt avlägsnande av tjocktarmen) och 22% (95% CI 15%-28%) av CD patienterna hade blivit opererade i buken. Jämfört med andra populationsbaserade kohorter (bestående

av såväl patienter som insjuknat under barnaåren som i vuxen ålder) var inflammationsutbredningen relativt stabil över tid och relativt få patienter kom att utveckla komplicerad sjukdom eller krävde bukkirurgi. De patienter som vid endoskopisk kontroll ett år efter diagnos uppvisade en kvarstående sårig tarmslemhinna hade en ökad risk för att drabbas av ett komplicerat sjukdomsförlopp (hazard kvot 14.56, 95% CI 1,79–118,68). Vår studie bekräftar att de patienter som insjuknar i IBD under barn- och ungdomsåren utmärks av en utbredd tarminflammation, men ifrågasätter hypotesen om att de som insjuknar i unga år, skulle vara drabbade av en mer dynamisk och aggressiv form av sjukdomen än de som insjuknar i vuxen ålder. Den starkare kopplingen mellan en oläkt tarmslemhinneinflammation än mellan betydande symptom ett år efter diagnos och ett komplicerat sjukdomsförlopp visar att intensiteten i underhållsbehandlingen vid CD även bör vägledas av endoskopiska fynd.

Denna avhandling bidrar med betydelsefulla kunskaper om incidensen av, riskfaktorer för och prognosen vid IBD hos barn och ungdomar vilka bör användas som utgångspunkt för framtida forskningsprojekt inom området.

12 ACKNOWLEDGEMENTS

I would like to express my gratitude to the following persons and institutions:

All the children and their parents who have taught me what paediatric IBD really represents and thus have given substantial meaning to my quest to increase the knowledge of IBD in order to ease the disease burden of children afflicted with IBD.

Scott Montgomery, whose imposing knowledge of epidemiology and brilliance in the British English has turned my unpolished manuscripts into papers that shine. Once this thesis is history I hope we will have more time to discuss other interests we share such as the global threat to amphibians and the forgotten poets of Närke.

Hans Hildebrand, my supervisor and clinical mentor and to some extent also role model in how to handle the constant conflict between working and living.

Lena Grahnquist, my supervisor and boss, who taught me that "good enough" is the aim to strive for rather than excellence (which comes with time anyway) and who provided working conditions that allowed me to write this thesis.

Bengt Kristiansson, my external mentor in research, who has taught me that the world is larger than IBD-epidemiology and that it is possible for a retired paediatrician to start an academic career in oriental language and literature and thus has nourished my dreams of what to do when I grow old.

Henrik Arnell, my room-mate, who with his never-ending drive to improve things has taught me how to handle references, layout and obsessive thinking. Throughout this work I have tried to follow his example of fixing things today so as to have time tomorrow for better things.

Maja Ideström, who by setting a living example taught me that producing an excellent dissertation on paediatric IBD doesn't mean you have to walk through purgatory.

Antal Nemeth, my former boss, who has inspired me to view the practice of clinical gastroenterology as a perpetual intellectual pursuit for which research methodology could provide you with the tools to dig further and deeper.

Karl Anders Dahlström, Björn Fischler, Jan Ejderhamn, and Thomas Casswall who each in their own way have guided me along my rather straight road to clinical paediatric gastroenterology but also have supported me along my more winding path to becoming a researcher.

To all my colleagues throughout the years who have taught I taught me both gastroenterology and the importance of comradeship.

Johan Lindholm, Ragnar Befrits, Jan Björck and Shahram Bamanyar, my co-authors, who have contributed not only with their individual expertise and experience but also with their dedication and infinite curiosity.

To Kajsa Nyquist, and all co-workers at the department of paediatric gastroenterology unit, for being, intelligent and empathetic professionals providing excellent care to the children with IBD. By also being flexible and understanding and skilled in logistics you have eased my clinical burden for a while and thus enabled me to combine increasing research with decreasing clinical work without anyone actually noticing that I was gone.

Yigael Finkel, whose organisational skills have a great part in the making of the paediatric gastroenterology department of ALB to what it is today and who throughout my work has contributed with critical comments and advice on how to present data in the most palatable way.

Anders Ekbom who started the research school of epidemiology and who has been one of the main contributors in making the field of IBD epidemiology a source of national pride.

To Astrid Häggblad and Anci Adestam at KI who have taught me that to do good research you should not only think free but first and foremost have things in order.

Finally, my deepest gratitude goes to my wife and our children, my extended family and my friends, for being there and for helping me to keep things in perspective.

Stiftelsen Samariten, Jerringstiftelsen, Stiftelsen Konung Gustaf VI Adolfs Frimurarefond, Broad Foundation's Broad Medical Research Program, Department of Women's and Children's Health Karolinska Institutet, Clinical Epidemiology Unit, Department of Medicine, Karolinska Institutet who provided the financial support that has made this work possible.

13 REFERENCES

1. Baron JH. Inflammatory bowel disease up to 1932. Mt Sinai J Med. 2000 May;67(3):174-89.
2. Wilks S. The morbid appearance of the intestine of Miss Banks. Medical Times and Gazette. 1859;2:264-5.
3. Dalziel TK. Chronic interstitial enteritis. BMJ. 1913;11:1068-70.
4. Lesniowski A. Przyczynek do chirurgii kiszek. Medycyna. 1903;31:460-518.
5. Moschowitz E, Wilensky AO. Non-specific granulomata of the intestine. Am J Med Sci. 1923;166:48-66.
6. Crohn BB, Ginzburg L, Oppenheimer GD. Regional ileitis: a pathologic and clinical entity. JAMA. 1932;99:1323-9.
7. Lockhart-Mummery HE, Morson BC. Crohn's disease (regional enteritis) of the large intestine and its distinction from ulcerative colitis. Gut. 1960 Jun;1:87-105.
8. Hadfield G. The primary histological lesion of regional ileitis. Lancet. 1939;2:773-5.
9. Turner D, Levine A, Escher JC, Griffiths AM, Russell RK, Dignass A, et al. Management of Pediatric Ulcerative Colitis: Joint ECCO and ESPGHAN Evidence-based Consensus Guidelines. J Pediatr Gastroenterol Nutr. 2012;55(3):340-61.
10. Van Assche G, Dignass A, Panes J, Beaugerie L, Karagiannis J, Allez M, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis. J Crohns Colitis. 2010;4(1):7-27.
11. Dignass A, Eliakim R, Magro F, Maaser C, Chowers Y, Geboes K, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 1: definitions and diagnosis. J Crohns Colitis. 2012 Dec;6(10):965-90.
12. Lennard-Jones JE. Classification of inflammatory bowel disease. Scand J Gastroenterol Suppl. 1989;170:2-6.
13. Langholz E. Ulcerative colitis. An epidemiological study based on a regional inception cohort, with special reference to disease course and prognosis. Dan Med Bull. 1999 Nov;46(5):400-15.
14. Munkholm P. Crohn's disease-occurrence, course and prognosis. An epidemiologic cohort-study. Dan Med Bull. 1997 Jun;44(3):287-302.
15. Inflammatory bowel disease in children and adolescents: recommendations for diagnosis--the Porto criteria. J Pediatr Gastroenterol Nutr. 2005 Jul;41(1):1-7.
16. Hildebrand H, Karlberg J, Kristiansson B. Longitudinal growth in children and adolescents with inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 1994 Feb;18(2):165-73.
17. Henriksen M, Jahnsen J, Lygren I, Sauar J, Schulz T, Stray N, et al. Change of diagnosis during the first five years after onset of inflammatory bowel disease: results of a prospective follow-up study (the IBSEN Study). Scand J Gastroenterol. 2006 Sep;41(9):1037-43.
18. Malaty HM, Mehta S, Abraham B, Garnett EA, Ferry GD. The natural course of inflammatory bowel disease-indeterminate from childhood to adulthood: within a 25 year period. Clin Exp Gastroenterol. 2013 Jul 23;6:115-21.
19. Bousvaros A, Antonioli DA, Colletti RB, Dubinsky MC, Glickman JN, Gold BD, et al. Differentiating ulcerative colitis from Crohn disease in children and young adults: report of a working group of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the Crohn's and Colitis Foundation of America. J Pediatr Gastroenterol Nutr. 2007 May;44(5):653-74.
20. Öst Å, et al. Gastrointestinal patologi - colon/rectum (Dok.nr GE7). Stockholm: Swedish Society of Pathology; 2006.
21. Solberg IC, Lygren I, Jahnsen J, Aadland E, Hoie O, Cvancarova M, et al. Clinical course during the first 10 years of ulcerative colitis: results from a

population-based inception cohort (IBSEN Study). Scand J Gastroenterol. 2009;44(4):431-40.
22. Langholz E, Munkholm P, Davidsen M, Binder V. Course of ulcerative colitis: analysis of changes in disease activity over years. Gastroenterology. 1994 Jul;107(1):3-11.
23. Ekbom A, Helmick C, Zack M, Adami HO. Ulcerative colitis and colorectal cancer. A population-based study. N Engl J Med. 1990 Nov 1;323(18):1228-33.
24. Bernstein CN, Ng SC, Lakatos PL, Moum B, Loftus EV, Jr. A review of mortality and surgery in ulcerative colitis: milestones of the seriousness of the disease. Inflamm Bowel Dis. 2013 Aug;19(9):2001-10.
25. Solberg IC, Vatn MH, Hoie O, Stray N, Sauar J, Jahnsen J, et al. Clinical course in Crohn's disease: results of a Norwegian population-based ten-year follow-up study. Clin Gastroenterol Hepatol. 2007 Dec;5(12):1430-8.
26. Munkholm P, Langholz E, Davidsen M, Binder V. Disease activity courses in a regional cohort of Crohn's disease patients. Scand J Gastroenterol. 1995 Jul;30(7):699-706.
27. Cosnes J, Cattan S, Blain A, Beaugerie L, Carbonnel F, Parc R, et al. Long-term evolution of disease behavior of Crohn's disease. Inflamm Bowel Dis. 2002 Jul;8(4):244-50.
28. Stjernman H, Tysk C, Almer S, Strom M, Hjortswang H. Unfavourable outcome for women in a study of health-related quality of life, social factors and work disability in Crohn's disease. Eur J Gastroenterol Hepatol. 2011 Aug;23(8):671-9.
29. Canavan C, Abrams KR, Hawthorne B, Mayberry JF. Long-term prognosis in Crohn's disease: An epidemiological study of patients diagnosed more than 20 years ago in Cardiff. Aliment Pharmacol Ther. 2007 Jan 1;25(1):59-65.
30. Wolters FL, Russel MG, Sijbrandij J, Schouten LJ, Odes S, Riis L, et al. Crohn's disease: increased mortality 10 years after diagnosis in a Europe-wide population based cohort. Gut. 2006 Apr;55(4):510-8.
31. Hovde O, Kempski-Monstad I, Smastuen MC, Solberg IC, Henriksen M, Jahnsen J, et al. Mortality and causes of death in Crohn's disease: results from 20 years of follow-up in the IBSEN study. Gut. 2013 Jun 6;6:6.
32. Manninen P, Karvonen AL, Huhtala H, Rasmussen M, Salo M, Mustaniemi L, et al. Mortality in ulcerative colitis and Crohn's disease. A population-based study in Finland. J Crohns Colitis. 2012 Jun;6(5):524-8.
33. Farmer RG, Hawk WA, Turnbull RB, Jr. Clinical patterns in Crohn's disease: a statistical study of 615 cases. Gastroenterology. 1975 Apr;68(4 Pt 1):627-35.
34. Sachar DB, Andrews HA, Farmer RG. Proposed classification of patient subgroups in Crohn's disease Gastroenterol Int 1992;5:141–54.
35. Greenstein AJ, Lachman P, Sachar DB, Springhorn J, Heimann T, Janowitz HD, et al. Perforating and non-perforating indications for repeated operations in Crohn's disease: evidence for two clinical forms. Gut. 1988 May;29(5):588-92.
36. Gasche C, Scholmerich J, Brynskov J, D'Haens G, Hanauer SB, Irvine EJ, et al. A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. Inflamm Bowel Dis. 2000;6(1):8-15.
37. Polito JM, 2nd, Childs B, Mellits ED, Tokayer AZ, Harris ML, Bayless TM. Crohn's disease: influence of age at diagnosis on site and clinical type of disease. Gastroenterology. 1996 Sep;111(3):580-6.
38. Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut. 2006 Jun;55(6):749-53.
39. Louis E, Collard A, Oger AF, Degroote E, Aboul Nasr El Yafi FA, Belaiche J. Behaviour of Crohn's disease according to the Vienna classification: changing pattern over the course of the disease. Gut. 2001 Dec;49(6):777-82.
40. Levine A, Griffiths A, Markowitz J, Wilson DC, Turner D, Russell RK, et al. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. Inflamm Bowel Dis. 2011 Jun;17(6):1314-21.

41. Heyman MB, Kirschner BS, Gold BD, Ferry G, Baldassano R, Cohen SA, et al. Children with early-onset inflammatory bowel disease (IBD): analysis of a pediatric IBD consortium registry. J Pediatr. 2005 Jan;146(1):35-40.
42. Lazarev M, Huang C, Bitton A, Cho JH, Duerr RH, McGovern DP, et al. Relationship between proximal Crohn's disease location and disease behavior and surgery: a cross-sectional study of the IBD Genetics Consortium. Am J Gastroenterol. 2013 Jan;108(1):106-12.
43. Dubinsky MC, Kugathasan S, Mei L, Picornell Y, Nebel J, Wrobel I, et al. Increased immune reactivity predicts aggressive complicating Crohn's disease in children. Clin Gastroenterol Hepatol. 2008 Oct;6(10):1105-11.
44. Vermeire S. Towards a novel molecular classification of IBD. Dig Dis. 2012;30(4):425-7.
45. Vermeire S, Van Assche G, Rutgeerts P. Classification of inflammatory bowel disease: the old and the new. Curr Opin Gastroenterol. 2012 Jul;28(4):321-6.
46. Jayanthi V, Robinson RJ, Malathi S, Rani B, Balambal R, Chari S, et al. Does Crohn's disease need differentiation from tuberculosis? J Gastroenterol Hepatol. 1996 Feb;11(2):183-6.
47. Schumacher G, Kollberg B, Sandstedt B. A prospective study of first attacks of inflammatory bowel disease and infectious colitis. Histologic course during the 1st year after presentation. Scand J Gastroenterol. 1994 Apr;29(4):318-32.
48. Naser SA, Ghobrial G, Romero C, Valentine JF. Culture of Mycobacterium avium subspecies paratuberculosis from the blood of patients with Crohn's disease. Lancet. 2004 Sep 18-24;364(9439):1039-44.
49. Ljungh A, Eriksson M, Eriksson O, Henter JI, Wadstrom T. Shiga-like toxin production and connective tissue protein binding of Escherichia coli isolated from a patient with ulcerative colitis. Scand J Infect Dis. 1988;20(4):443-6.
50. Montgomery SM, Morris DL, Pounder RE, Wakefield AJ. Paramyxovirus infections in childhood and subsequent inflammatory bowel disease. Gastroenterology. 1999 Apr;116(4):796-803.
51. Nystrom N, Berg T, Lundin E, Skog O, Hansson I, Frisk G, et al. Human enterovirus species B in ileocecal Crohn's disease. Clin Transl Gastroenterol. 2013 Jun 27;4:e38.
52. Jarnerot G, Rolny P, Wickbom G, Alemayehu G. Antimycobacterial therapy ineffective in Crohn's disease after a year. Lancet. 1989 Jan 21;1(8630):164-5.
53. Halfvarson J, Jess T, Bodin L, Jarnerot G, Munkholm P, Binder V, et al. Longitudinal concordance for clinical characteristics in a Swedish-Danish twin population with inflammatory bowel disease. Inflamm Bowel Dis. 2007 Dec;13(12):1536-44.
54. Halfvarson J, Jess T, Magnuson A, Montgomery SM, Orholm M, Tysk C, et al. Environmental factors in inflammatory bowel disease: a co-twin control study of a Swedish-Danish twin population. Inflamm Bowel Dis. 2006 Oct;12(10):925-33.
55. Begue B, Verdier J, Rieux-Laucat F, Goulet O, Morali A, Canioni D, et al. Defective IL10 signaling defining a subgroup of patients with inflammatory bowel disease. Am J Gastroenterol. 2011 Aug;106(8):1544-55.
56. Lees CW, Barrett JC, Parkes M, Satsangi J. New IBD genetics: common pathways with other diseases. Gut. 2011;7:7.
57. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature. 2001 May 31;411(6837):599-603.
58. Parkes M, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA, et al. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. Nat Genet. 2007;39(7):830-2.
59. Barrett JC, Lee JC, Lees CW, Prescott NJ, Anderson CA, Phillips A, et al. Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. Nat Genet. 2009;41(12):1330-4.
60. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology. 2012 Jan;142(1):46-54.

61. Vind I, Riis L, Jess T, Knudsen E, Pedersen N, Elkjaer M, 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 Jun;101(6):1274-82.
62. Jussila A, Virta LJ, Kautiainen H, Rekiaro M, Nieminen U, Farkkila MA. Increasing incidence of inflammatory bowel diseases between 2000 and 2007: a nationwide register study in Finland. Inflamm Bowel Dis. 2012 Mar;18(3):555-61.
63. Sjoberg D, Holmstrom T, Larsson M, Nielsen AL, Holmquist L, Ekbom A, et al. Incidence and natural history of ulcerative colitis in the Uppsala Region of Sweden 2005-2009 - results from the IBD cohort of the Uppsala Region (ICURE). J Crohns Colitis. 2013 Oct 1;7(9):e351-7.
64. Yang SK, Yun S, Kim JH, Park JY, Kim HY, Kim YH, et al. Epidemiology of inflammatory bowel disease in the Songpa-Kangdong district, Seoul, Korea, 1986-2005: a KASID study. Inflamm Bowel Dis. 2008 Apr;14(4):542-9.
65. Ekbom A. The epidemiology of IBD: a lot of data but little knowledge. How shall we proceed? Inflamm Bowel Dis. 2004;10(Suppl 1):S32-4.
66. Ekbom A, Helmick C, Zack M, Adami HO. The epidemiology of inflammatory bowel disease: a large, population-based study in Sweden. Gastroenterology. 1991 Feb;100(2):350-8.
67. Norlen BJ, Krause U, Bergman L. An epidemiological study of Crohn's disease. Scand J Gastroenterol. 1970;5(5):385-90.
68. Munkholm P, Langholz E, Nielsen OH, Kreiner S, Binder V. Incidence and prevalence of Crohn's disease in the county of Copenhagen, 1962-87: a sixfold increase in incidence. Scand J Gastroenterol. 1992 Jul;27(7):609-14.
69. Benchimol EI, Fortinsky KJ, Gozdyra P, Van den Heuvel M, Van Limbergen J, Griffiths AM. Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. Inflamm Bowel Dis. 2011 Jan;17(1):423-39.
70. Braegger CP, Ballabeni P, Rogler D, Vavricka SR, Friedt M, Pittet V, et al. Epidemiology of Inflammatory Bowel Disease: Is There a Shift Towards Onset at a Younger Age? J Pediatr Gastroenterol Nutr. 2011 Aug;53(2):141-4.
71. Hildebrand H, Finkel Y, Grahnquist L, Lindholm J, Ekbom A, Askling J. Changing pattern of paediatric inflammatory bowel disease in northern Stockholm 1990-2001. Gut. 2003 Oct;52(10):1432-4.
72. Lehtinen P, Ashorn M, Iltanen S, Jauhola R, Jauhonen P, Kolho KL, et al. Incidence trends of pediatric inflammatory bowel disease in Finland, 1987-2003, a nationwide study. Inflamm Bowel Dis. 2011;17(8):1778-83.
73. Benchimol EI, Guttmann A, Griffiths AM, Rabeneck L, Mack DR, Brill H, et al. Increasing incidence of paediatric inflammatory bowel disease in Ontario, Canada: evidence from health administrative data. Gut. 2009;58(11):1490-7.
74. Gent AE, Hellier MD, Grace RH, Swarbrick ET, Coggon D. Inflammatory bowel disease and domestic hygiene in infancy. Lancet. 1994 Mar 26;343(8900):766-7.
75. Sonnenberg A. Occupational distribution of inflammatory bowel disease among German employees. Gut. 1990 Sep;31(9):1037-40.
76. Mayberry JF, Dew MJ, Morris JS, Powell DB. An audit of Crohn's disease in a defined population Journal of the Royal College of Physicians of London 1983;17(3):196-8.
77. Gearry RB, Richardson AK, Frampton CM, Dodgshun AJ, Barclay ML. Population-based cases control study of inflammatory bowel disease risk factors. J Gastroenterol Hepatol. 2010 Feb;25(2):325-33.
78. Ehlin AG, Montgomery SM, Ekbom A, Pounder RE, Wakefield AJ. Prevalence of gastrointestinal diseases in two British national birth cohorts. Gut. 2003 Aug;52(8):1117-21.
79. Gilat T, Hacohen D, Lilos P, Langman MJ. Childhood factors in ulcerative colitis and Crohn's disease. An international cooperative study. Scand J Gastroenterol. 1987 Oct;22(8):1009-24.

80. Abramson O, Durant M, Mow W, Finley A, Kodali P, Wong A, et al. Incidence, prevalence, and time trends of pediatric inflammatory bowel disease in Northern California, 1996 to 2006. J Pediatr. 2010 Aug;157(2):233-9.
81. Montgomery SM, Morris DL, Pounder RE, Wakefield AJ. Asian ethnic origin and the risk of inflammatory bowel disease. Eur J Gastroenterol Hepatol. 1999 May;11(5):543-6.
82. Tysk C, Lindberg E, Jarnerot G, Floderus-Myrhed B. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. Gut. 1988 Jul;29(7):990-6.
83. Cornish JA, Tan E, Simillis C, Clark SK, Teare J, Tekkis PP. The risk of oral contraceptives in the etiology of inflammatory bowel disease: a meta-analysis. Am J Gastroenterol. 2008 Sep;103(9):2394-400.
84. Ekbom A, Adami HO, Helmick CG, Jonzon A, Zack MM. Perinatal risk factors for inflammatory bowel disease: a case-control study. Am J Epidemiol. 1990 Dec;132(6):1111-9.
85. Wurzelmann JI, Lyles CM, Sandler RS. Childhood infections and the risk of inflammatory bowel disease. Dig Dis Sci. 1994 Mar;39(3):555-60.
86. Barclay AR, Russell RK, Wilson ML, Gilmour WH, Satsangi J, Wilson DC. Systematic review: the role of breastfeeding in the development of pediatric inflammatory bowel disease. J Pediatr. 2009 Sep;155(3):421-6.
87. Soderhjelm L, Zetterstrom R. Trends in infant feeding in Sweden during the 20th century. Acta Paediatr Scand Suppl. 1990;365:5-6.
88. Strachan DP. Hay fever, hygiene, and household size. BMJ. 1989 Nov 18;299(6710):1259-60.
89. Rook GA. Hygiene hypothesis and autoimmune diseases. Clin Rev Allergy Immunol. 2012 Feb;42(1):5-15. PubMed PMID: 22090147.
90. Montgomery SM, Lambe M, Wakefield AJ, Pounder RE, Ekbom A. Siblings and the risk of inflammatory bowel disease. Scand J Gastroenterol. 2002 Nov;37(11):1301-8. PubMed PMID: 12465729.
91. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A. 2010 Aug 17;107(33):14691-6.
92. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012 Nov 1;491(7422):119-24.
93. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol. 2009 May;9(5):313-23. PubMed PMID: 19343057. Epub 2009/04/04. eng.
94. Wold AE AI. Pathological consequences of commensalism. In: Nataro JP BM, Cunningham-Rundles S, editor. Persistent bacterial infections. Washington , DC ASM Press; 2000. p. 115-44.
95. Rutgeerts P, Goboes K, Peeters M, Hiele M, Penninckx F, Aerts R, et al. Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. Lancet. 1991 Sep 28;338(8770):771-4.
96. Cobrin GM, Abreu MT. Defects in mucosal immunity leading to Crohn's disease. Immunol Rev. 2005 Aug;206:277-95.
97. Ellis-Pegler RB, Crabtree C, Lambert HP. The faecal flora of children in the United Kingdom. J Hyg (Lond). 1975 Aug;75(1):135-42.
98. Adlerberth I, Lindberg E, Aberg N, Hesselmar B, Saalman R, Strannegard IL, et al. Reduced enterobacterial and increased staphylococcal colonization of the infantile bowel: an effect of hygienic lifestyle? Pediatr Res. 2006 Jan;59(1):96-101.
99. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux JJ, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A. 2008 Oct 28;105(43):16731-6.

100. Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. Lancet. 2003 May 31;361(9372):1869-71.
101. Favier CF, Vaughan EE, De Vos WM, Akkermans AD. Molecular monitoring of succession of bacterial communities in human neonates. Appl Environ Microbiol. 2002 Jan;68(1):219-26.
102. Bedford Russell AR, Murch SH. Could peripartum antibiotics have delayed health consequences for the infant? BJOG. 2006 Jul;113(7):758-65.
103. Salminen S, Gibson GR, McCartney AL, Isolauri E. Influence of mode of delivery on gut microbiota composition in seven year old children. Gut. 2004 Sep;53(9):1388-9.
104. Svartz N. The treatment of ulcerative colitis. Gastroenterologia. 1956;86(5):683-8.
105. Dick AP, Grayson MJ, Carpenter RG, Petrie A. Controlled Trial of Sulphasalazine in the Treatment of Ulcerative Colitis. Gut. 1964 Oct;5:437-42.
106. Truelove SC, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. Br Med J. 1955 Oct 29;2(4947):1041-8.
107. Watts JM, De Dombal FT, Watkinson G, Goligher JC. Long-term prognosis of ulcerative colitis. Br Med J. 1966 Jun 11;1(5501):1447-53.
108. Vermeire S, van Assche G, Rutgeerts P. Review article: Altering the natural history of Crohn's disease--evidence for and against current therapies. Aliment Pharmacol Ther. 2007 Jan 1;25(1):3-12.
109. Jess T, Riis L, Vind I, Winther KV, Borg S, Binder V, et al. Changes in clinical characteristics, course, and prognosis of inflammatory bowel disease during the last 5 decades: a population-based study from Copenhagen, Denmark. Inflamm Bowel Dis. 2007 Apr;13(4):481-9.
110. Jess T, Simonsen J, Jorgensen KT, Pedersen BV, Nielsen NM, Frisch M. Decreasing risk of colorectal cancer in patients with inflammatory bowel disease over 30 years. Gastroenterology. 2012;143(2):375-81.
111. Cosnes J, Nion-Larmurier I, Beaugerie L, Afchain P, Tiret E, Gendre JP. Impact of the increasing use of immunosuppressants in Crohn's disease on the need for intestinal surgery. Gut. 2005 Feb;54(2):237-41.
112. Griffiths AM. Specificities of inflammatory bowel disease in childhood. Best Pract Res Clin Gastroenterol. 2004 Jun;18(3):509-23.
113. Jakobsen C, Bartek J, Wewer V, Vind I, Munkholm P, Groen R, et al. Differences in phenotype and disease course in adult and paediatric inflammatory bowel disease--a population-based study. Aliment Pharmacol Ther. 2011;34(10):1217-24.
114. Van Limbergen J, Russell RK, Drummond HE, Aldhous MC, Round NK, Nimmo ER, et al. Definition of phenotypic characteristics of childhood-onset inflammatory bowel disease. Gastroenterology. 2008 Oct;135(4):1114-22.
115. Langholz E, Munkholm P, Krasilnikoff PA, Binder V. Inflammatory bowel diseases with onset in childhood. Clinical features, morbidity, and mortality in a regional cohort. Scand J Gastroenterol. 1997 Feb;32(2):139-47.
116. Vernier-Massouille G, Balde M, Salleron J, Turck D, Dupas JL, Mouterde O, et al. Natural history of pediatric Crohn's disease: a population-based cohort study. Gastroenterology. 2008 Oct;135(4):1106-13.
117. Gower-Rousseau C, Dauchet L, Vernier-Massouille G, Tilloy E, Brazier F, Merle V, et al. The natural history of pediatric ulcerative colitis: a population-based cohort study. Am J Gastroenterol. 2009 Aug;104(8):2080-8.
118. Jakobsen C, Paerregaard A, Munkholm P, Wewer V. 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 Nov;21(11):1291-301.
119. Walters TD, Gilman AR, Griffiths AM. Linear growth improves during infliximab therapy in children with chronically active severe Crohn's disease. Inflamm Bowel Dis. 2007 Apr;13(4):424-30.

120. Mowat C, Cole A, Windsor A, Ahmad T, Arnott I, Driscoll R, et al. Guidelines for the management of inflammatory bowel disease in adults. Gut. 2011 May;60(5):571-607.
121. Turner D, Otley AR, Mack D, Hyams J, de Bruijne J, Uusoue K, et al. Development, validation, and evaluation of a pediatric ulcerative colitis activity index: a prospective multicenter study. Gastroenterology. 2007 Aug;133(2):423-32.
122. D'Haens G, Ferrante M, Vermeire S, Baert F, Noman M, Moortgat L, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflamm Bowel Dis. 2012 Dec;18(12):2218-24.
123. Froslie KF, Jahnsen J, Moum BA, Vatn MH. Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort. Gastroenterology. 2007 Aug;133(2):412-22.
124. Allez M, Lemann M. Role of endoscopy in predicting the disease course in inflammatory bowel disease. World J Gastroenterol. 2010 Jun 7;16(21):2626-32.
125. Loly C, Belaiche J, Louis E. Predictors of severe Crohn's disease. Scand J Gastroenterol. 2008 Aug;43(8):948-54.
126. Beaugerie L, Seksik P, Nion-Larmurier I, Gendre JP, Cosnes J. Predictors of Crohn's disease. Gastroenterology. 2006 Mar;130(3):650-6.
127. Hildebrand H, Brydolf M, Holmquist L, Krantz I, Kristiansson B. Incidence and prevalence of inflammatory bowel disease in children in south-western Sweden. Acta Paediatr. 1994 Jun;83(6):640-5.
128. Lindberg E, Lindquist B, Holmquist L, Hildebrand H. Inflammatory bowel disease in children and adolescents in Sweden, 1984-1995. J Pediatr Gastroenterol Nutr. 2000 Mar;30(3):259-64.
129. Hildebrand H, Fredrikzon B, Holmquist L, Kristiansson B, Lindquist B. Chronic inflammatory bowel disease in children and adolescents in Sweden. J Pediatr Gastroenterol Nutr. 1991 Oct;13(3):293-7.
130. Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. BMC Public Health. 2011 Jun 9;11:450.
131. Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, Ekbom A. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. Eur J Epidemiol. 2009;24(11):659-67.
132. Pundziute-Lycka A, Dahlquist G, Nystrom L, Arnqvist H, Bjork E, Blohme G, et al. The incidence of Type I diabetes has not increased but shifted to a younger age at diagnosis in the 0-34 years group in Sweden 1983-1998. Diabetologia. 2002 Jun;45(6):783-91.
133. Harjutsalo V, Sjoberg L, Tuomilehto J. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. Lancet. 2008 May 24;371(9626):1777-82.
134. Gopinath S, Ortqvist E, Norgren S, Green A, Sanjeevi CB. Variations in incidence of type 1 diabetes in different municipalities of stockholm. Ann N Y Acad Sci. 2008 Dec;1150:200-7.
135. Sjoberg D, Holmstrom T, Larsson M, Nielsen AL, Holmquist L, Ekbom A, et al. Incidence and clinical course of Crohn's disease during the first year - Results from the IBD Cohort of the Uppsala Region (ICURE) of Sweden 2005-2009. J Crohns Colitis. 2013 Sep 11;11(13):00280-8.
136. Levine A, Koletzko S, Turner D, Escher JC, Cucchiara S, de Ridder L, et al. The ESPGHAN Revised Porto Criteria for the Diagnosis of Inflammatory Bowel Disease in Children and Adolescents. J Pediatr Gastroenterol Nutr. 2013 Nov 13;13:13.
137. Perminow G, Frigessi A, Rydning A, Nakstad B, Vatn MH. Incidence and clinical presentation of IBD in children: comparison between prospective and retrospective data in a selected Norwegian population. Scand J Gastroenterol. 2006 Dec;41(12):1433-9.
138. Jakobsen C, Paerregaard A, Munkholm P, Faerk J, Lange A, Andersen J, 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;17(12):2541-50.
139. Molstad S, Erntell M, Hanberger H, Melander E, Norman C, Skoog G, et al. Sustained reduction of antibiotic use and low bacterial resistance: 10-year follow-up of the Swedish Strama programme. Lancet Infect Dis. 2008 Feb;8(2):125-32.
140. Virta L, Auvinen A, Helenius H, Huovinen P, Kolho KL. Association of repeated exposure to antibiotics with the development of pediatric Crohn's disease--a nationwide, register-based finnish case-control study. Am J Epidemiol. 2012 Apr 15;175(8):775-84.
141. Hviid A, Svanstrom H, Frisch M. Antibiotic use and inflammatory bowel diseases in childhood. Gut. 2011 Jan;60(1):49-54.
142. Bager P, Simonsen J, Nielsen NM, Frisch M. Cesarean section and offspring's risk of inflammatory bowel disease: a national cohort study. Inflamm Bowel Dis. 2012 May;18(5):857-62.
143. Wiklund I, Edman G, Andolf E. Cesarean section on maternal request: reasons for the request, self-estimated health, expectations, experience of birth and signs of depression among first-time mothers. Acta Obstet Gynecol Scand. 2007;86(4):451-6.
144. Odlind V, Haglund B, Pakkanen M, Otterblad Olausson P. Deliveries, mothers and newborn infants in Sweden, 1973-2000. Trends in obstetrics as reported to the Swedish Medical Birth Register. Acta Obstet Gynecol Scand. 2003 Jun;82(6):516-28.
145. Malmborg P, Grahnquist L, Lindholm J, Montgomery S, Hildebrand H. Increasing incidence of paediatric inflammatory bowel disease in northern Stockholm County, 2002-2007. J Pediatr Gastroenterol Nutr. 2013 Jul;57(1):29-34.
146. Martin-de-Carpi J, Rodriguez A, Ramos E, Jimenez S, Martinez-Gomez MJ, Medina E. Increasing incidence of pediatric inflammatory bowel disease in Spain (1996-2009): the SPIRIT Registry. Inflamm Bowel Dis. 2013 Jan;19(1):73-80.
147. Molstad S, Lundborg CS, Karlsson AK, Cars O. Antibiotic prescription rates vary markedly between 13 European countries. Scand J Infect Dis. 2002;34(5):366-71.
148. Perminow G, Brackmann S, Lyckander LG, Franke A, Borthne A, Rydning A, et al. A characterization in childhood inflammatory bowel disease, a new population-based inception cohort from South-Eastern Norway, 2005-07, showing increased incidence in Crohn's disease. Scand J Gastroenterol. 2009;44(4):446-56.
149. Pigneur B, Seksik P, Viola S, Viala J, Beaugerie L, Girardet JP, et al. Natural history of Crohn's disease: comparison between childhood- and adult-onset disease. Inflamm Bowel Dis. 2010 Jun;16(6):953-61.
150. Lovasz BD, Lakatos L, Horvath A, Szita I, Pandur T, Mandel M, et al. Evolution of disease phenotype in adult and pediatric onset Crohn's disease in a population-based cohort. World J Gastroenterol. 2013 Apr 14;19(14):2217-26.
151. Tarrant KM, Barclay ML, Frampton CM, Gearry RB. Perianal disease predicts changes in Crohn's disease phenotype-results of a population-based study of inflammatory bowel disease phenotype. Am J Gastroenterol. 2008 Dec;103(12):3082-93.
152. Rungoe C, Langholz E, Andersson M, Basit S, Nielsen NM, Wohlfahrt J, et al. Changes in medical treatment and surgery rates in inflammatory bowel disease: a nationwide cohort study 1979-2011. Gut. 2013 Sep 20;20(10):2013-305607.
153. Israeli E, Ryan JD, Shafer LA, Bernstein CN. Younger age at diagnosis is associated with panenteric, but not more aggressive, Crohn's disease. Clin Gastroenterol Hepatol. 2014 Jan;12(1):72-9.
154. Allen PB, Peyrin-Biroulet L. Moving towards disease modification in inflammatory bowel disease therapy. Curr Opin Gastroenterol. 2013 Jul;29(4):397-404.
155. Smolen JS, Landewe R, Breedveld FC, Dougados M, Emery P, Gaujoux-Viala C, et al. EULAR recommendations for the management of rheumatoid arthritis with

synthetic and biological disease-modifying antirheumatic drugs. Ann Rheum Dis. 2010 Jun;69(6):964-75.
156. Rantalaiho V, Korpela M, Laasonen L, Kautiainen H, Jarvenpaa S, Hannonen P, et al. Early combination disease-modifying antirheumatic drug therapy and tight disease control improve long-term radiologic outcome in patients with early rheumatoid arthritis: the 11-year results of the Finnish Rheumatoid Arthritis Combination Therapy trial. Arthritis Res Ther. 2010;12(3):R122.
157. Felson DT, Smolen JS, Wells G, Zhang B, van Tuyl LH, Funovits J, et al. American College of Rheumatology/European League against Rheumatism provisional definition of remission in rheumatoid arthritis for clinical trials. Ann Rheum Dis. 2011 Mar;70(3):404-13.
158. Zallot C, Peyrin-Biroulet L. Deep remission in inflammatory bowel disease: looking beyond symptoms. Curr Gastroenterol Rep. 2013 Mar;15(3):315.
159. Baert F, Moortgat L, Van Assche G, Caenepeel P, Vergauwe P, De Vos M, et al. Mucosal healing predicts sustained clinical remission in patients with early-stage Crohn's disease. Gastroenterology. 2010 Feb;138(2):463-8.
160. Colombel JF, Rutgeerts PJ, Sandborn WJ, Yang M, Camez A, Pollack PF, et al. Adalimumab Induces Deep Remission in Patients With Crohn's Disease. Clin Gastroenterol Hepatol. 2013 Jul 12;12(13):00924-5.
161. Pariente B, Cosnes J, Danese S, Sandborn WJ, Lewin M, Fletcher JG, et al. Development of the Crohn's disease digestive damage score, the Lemann score. Inflamm Bowel Dis. 2011 Jun;17(6):1415-22.
162. Koevoets R, Dirven L, Klarenbeek NB, van Krugten MV, Ronday HK, van der Heijde DM, et al. 'Insights in the relationship of joint space narrowing versus erosive joint damage and physical functioning of patients with RA'. Ann Rheum Dis. 2013 Jun;72(6):870-4.
163. Peyrin-Biroulet L, Cieza A, Sandborn WJ, Coenen M, Chowers Y, Hibi T, et al. Development of the first disability index for inflammatory bowel disease based on the international classification of functioning, disability and health. Gut. 2012 Feb;61(2):241-7.
164. Askling J, Baecklund E, Granath F, Geborek P, Fored M, Backlin C, et al. Anti-tumour necrosis factor therapy in rheumatoid arthritis and risk of malignant lymphomas: relative risks and time trends in the Swedish Biologics Register. Ann Rheum Dis. 2009 May;68(5):648-53.
165. Mackey AC, Green L, Leptak C, Avigan M. Hepatosplenic T cell lymphoma associated with infliximab use in young patients treated for inflammatory bowel disease: update. J Pediatr Gastroenterol Nutr. 2009 Mar;48(3):386-8.
166. Visser K, Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Ronday HK, Seys PE, Kerstens PJ, et al. A matrix risk model for the prediction of rapid radiographic progression in patients with rheumatoid arthritis receiving different dynamic treatment strategies: post hoc analyses from the BeSt study. Ann Rheum Dis. 2010 Jul;69(7):1333-7.
167. Savoye G, Salleron J, Gower-Rousseau C, Dupas JL, Vernier-Massouille G, Fumery M, et al. Clinical predictors at diagnosis of disabling pediatric Crohn's disease. Inflamm Bowel Dis. 2012 Nov;18(11):2072-8.
168. Allez M, Lemann M, Bonnet J, Cattan P, Jian R, Modigliani R. Long term outcome of patients with active Crohn's disease exhibiting extensive and deep ulcerations at colonoscopy. Am J Gastroenterol. 2002 Apr;97(4):947-53.
169. Huttenhower C, Gevers D, Knight R, al. e. Structure, function and diversity of the healthy human microbiome. Nature. 2012 Jun 14;486(7402):207-14.
170. Nwosu FC, Thorkildsen LT, Avershina E, Ricanek P, Perminow G, Brackmann S, et al. Age-dependent fecal bacterial correlation to inflammatory bowel disease for newly diagnosed untreated children. Gastroenterol Res Pract. 2013;2013:302398.
171. Sinagra E, Tomasello G, Cappello F, Leone A, Cottone M, Bellavia M, et al. Probiotics, prebiotics and symbiotics in inflammatory bowel diseases: state-of-the-art and new insights. J Biol Regul Homeost Agents. 2013 Oct-Dec;27(4):919-33.

172. Anderson JL, Edney RJ, Whelan K. Systematic review: faecal microbiota transplantation in the management of inflammatory bowel disease. Aliment Pharmacol Ther. 2012 Sep;36(6):503-16.
173. Kruger M, Shehata AA, Schrodl W, Rodloff A. Glyphosate suppresses the antagonistic effect of Enterococcus spp. on Clostridium botulinum. Anaerobe. 2013 Apr;20:74-8.
174. Dong L, Yan H, Wang D. Antibiotic prescribing patterns in village health clinics across 10 provinces of Western China. J Antimicrob Chemother. 2008 Aug;62(2):410-5.
175. Klemetti R, Che X, Gao Y, Raven J, Wu Z, Tang S, et al. Cesarean section delivery among primiparous women in rural China: an emerging epidemic. Am J Obstet Gynecol. 2010 Jan;202(1):65 e1-6.
176. Markowitz J, Grancher K, Kohn N, Lesser M, Daum F. A multicenter trial of 6-mercaptopurine and prednisone in children with newly diagnosed Crohn's disease. Gastroenterology. 2000;119(4):895-902.
177. Hyams J, Crandall W, Kugathasan S, Griffiths A, Olson A, Johanns J, et al. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. Gastroenterology. 2007 Mar;132(3):863-73.
178. Hyams J, Damaraju L, Blank M, Johanns J, Guzzo C, Winter HS, et al. Induction and maintenance therapy with infliximab for children with moderate to severe ulcerative colitis. Clin Gastroenterol Hepatol. 2012 Apr;10(4):391-9.
179. Crombe V, Salleron J, Savoye G, Dupas JL, Vernier-Massouille G, Lerebours E, et al. Long-term outcome of treatment with infliximab in pediatric-onset Crohn's disease: a population-based study. Inflamm Bowel Dis. 2011 Oct;17(10):2144-52.
180. Sandborn WJ, Gasink C, Gao LL, Blank MA, Johanns J, Guzzo C, et al. Ustekinumab induction and maintenance therapy in refractory Crohn's disease. N Engl J Med. 2012 Oct 18;367(16):1519-28.
181. Sandborn WJ, Feagan BG, Stoinov S, Honiball PJ, Rutgeerts P, Mason D, et al. Certolizumab pegol for the treatment of Crohn's disease. N Engl J Med. 2007 Jul 19;357(3):228-38.
182. Feagan BG, Rutgeerts P, Sands BE, Hanauer S, Colombel JF, Sandborn WJ, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. N Engl J Med. 2013 Aug 22;369(8):699-710.
183. Ashworth LA, Billett A, Mitchell P, Nuti F, Siegel C, Bousvaros A. Lymphoma risk in children and young adults with inflammatory bowel disease: analysis of a large single-center cohort. Inflamm Bowel Dis. 2012 May;18(5):838-43.
184. Solberg IC, Cvancarova M, Vatn MH, Moum B. Risk Matrix for Prediction of Advanced Disease in a Population-based Study of Patients with Crohn's Disease (the IBSEN Study). Inflamm Bowel Dis. 2014 Jan;20(1):60-8.
185. Molander P, af Bjorkesten CG, Mustonen H, Haapamaki J, Vauhkonen M, Kolho KL, et al. Fecal calprotectin concentration predicts outcome in inflammatory bowel disease after induction therapy with TNFalpha blocking agents. Inflamm Bowel Dis. 2012 Nov;18(11):2011-7.
186. Panaccione R, Colombel JF, Louis E, Peyrin-Biroulet L, Sandborn WJ. Evolving definitions of remission in Crohn's disease. Inflamm Bowel Dis. 2013 Jul;19(8):1645-53.
187. Louis E. Epidemiology of the transition from early to late Crohn's disease. Dig Dis. 2012;30(4):376-9.