Paediatric inflammatory bowel disease: scoping the future : genetics, diagnostics and therapeutics

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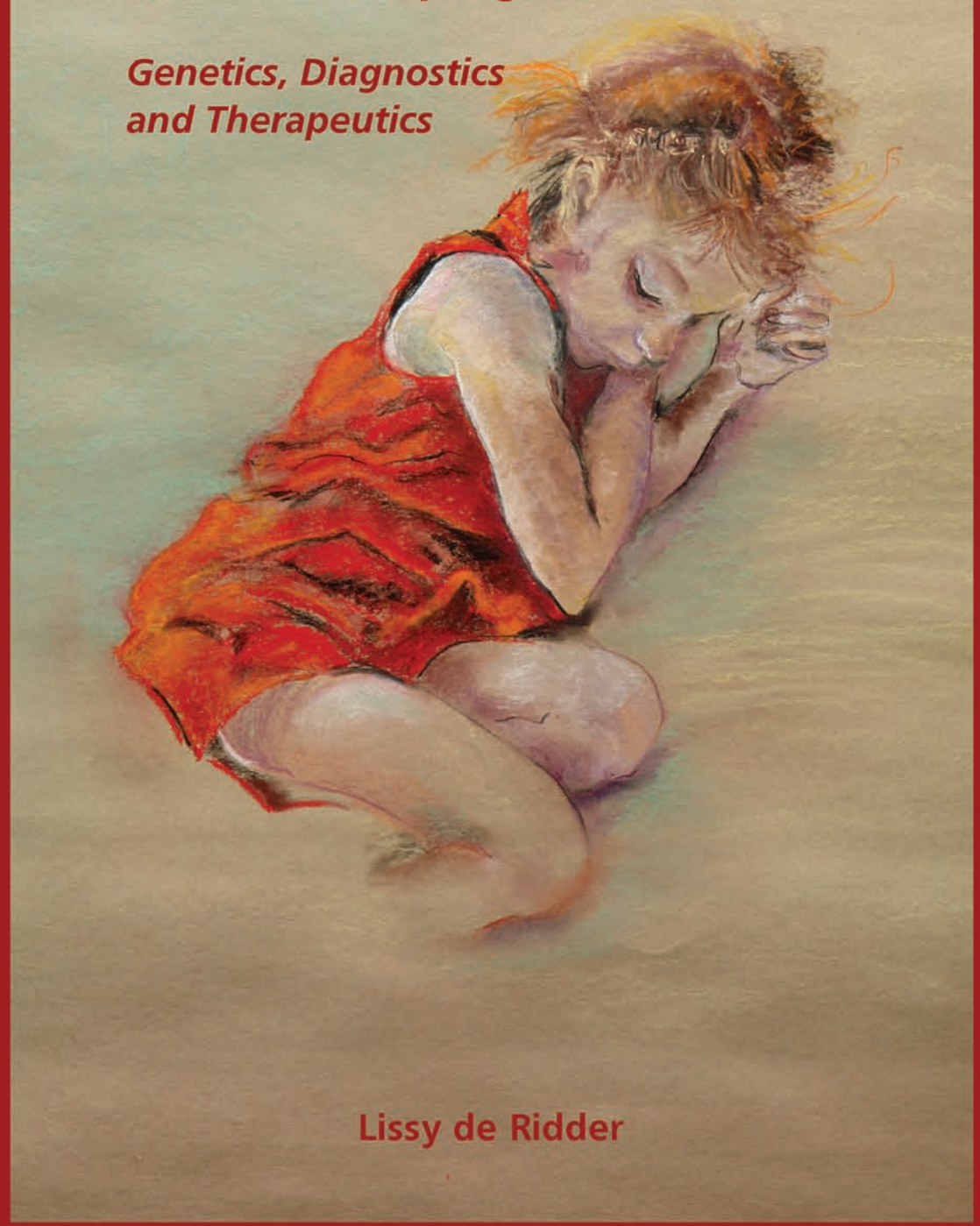

Lissy de Ridder

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Genetics, Diagnostics and Therapeutics

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Contents

Chapter 1

Introduction

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Paediatric Inflammatory Bowel Disease

Background

Crohn's Disease (CD), Ulcerative Colitis (UC) and indeterminate colitis (IC) are chronic inflammatory bowel diseases (IBD). CD is a segmental, transmural inflammation that can concern the whole digestive tract, from mouth to anus. The terminal ileum is the most commonly affected site. Presenting complaints often are abdominal pain, weight loss, diarrhoea and poor appetite. However, the classical triad of abdominal pain, weight loss, diarrhoea is present in only 25% of children with CD.(1) Many children with CD present with vague complaints, such as lethargy, anorexia and mild abdominal complaints.(1,2) Perianal complaints such as skin tags, fistulas and abscess formation can occur. Systemic complaints such as fatigue, fever and extraintestinal manifestations can occur. Osteoporosis can already be present when the disease is diagnosed. Growth failure and delay in puberty development are specific problems for paediatric CD and sometimes precede other symptoms.

UC mainly concerns inflammation of the mucosa, limited to the colon and rectum. In contrast to the segmental inflammation of CD, UC has continuous involvement, extending from the rectum proximally. Presenting complaints often are diarrhoea, rectal bleeding and abdominal pain. Sometimes systemic signs such as fever and weight loss are present. Osteoporosis, growth failure and delay in puberty development can occur, but much less frequent than in CD.

Sometimes it is not possible to make a distinction between CD and UC, because patients present with colitis, without specific features of either CD or UC. These patients are classified as having IC.

Epidemiology

In 20-25% of patients the disease presents before the age of 20. The incidence of paediatric IBD (under the age of 18) in The Netherlands is recently assessed and concerns 5.2/100.000 children.(3) In the UK and Republic of Ireland 5.2/100 000 children less than 16 years of age were newly diagnosed as having IBD. Sixty percent had CD, 28% had UC and 12% IC.(4) In South Wales comparable data were found.(5) The incidence has increased over the last 30-40 years.(6) In the seventies and eighties UC was diagnosed more frequently in children and adolescents, while nowadays CD is diagnosed more frequently. The cause of the rising incidence and changing disease pattern is unknown but may well be related to environmental factors playing a role in the aetiology of IBD.

Clinical features of paediatric IBD are different from adult IBD. In adults diarrhoea (CD) or rectal bleeding (UC) is the most frequently presenting symptom while in children this is abdominal pain. In adults UC is predominantly confined to the rectum or left-sided colon while children usually present with pancolitis.(1,7) Linear growth retardation and pubertal delay of course are very specific problems for the paediatric IBD population.(8)

Aetiology of IBD

IBD is a multi-factorial disorder with a complex interaction between immunological, genetic and bacterial factors, leading to inflammation of the gut and the gut mucosa.

CD is characterized by a T-helper type-1 (T_H1) immune response. Probably an abnormal reactivity against luminal and mucosal antigens gets pro-inflammatory and regulating CD_4^+-helper-T-lymfocytes out of balance. This causes a disturbance between T_H1-associated inflammatory cytokines on one hand, such as 'tumor necrosis factor-alpha' (TNF-α), interferon-gamma (IFN-γ), interleukin-12 (IL-12) and interleukin-18 (IL-18) and anti-inflammatory cytokines such as interleukin-10 (IL-10) and 'transforming growth factor-beta' (TGF-β) on the other hand. In UC evidence suggests that an excessive T helper 2-cell response occurs rather than the T_h1-cell pattern typical of CD.(9)

During the last years many factors of aetiology and pathophysiology in IBD are elucidated. Epidemiological studies have shown the important role of genetic factors in the development of IBD. Prevalence of IBD differs by race and ethnicity. In monozygotic twins a higher concordance is found than in dizygotic twins. There is also clustering of IBD within families.

About 5% -10% of IBD patients have a positive family history for IBD.(10,11) A positive family history therefore is the most important risk factor for the development of IBD. The probability to develop IBD before the age of 30 years when both parents are known with IBD is about 33%.(12) The relative risk to develop IBD in a sibling of an IBD patient is estimated between 15 en 35 for CD and between 7 and 17 for UC.(12,13) The genetic influence in CD is stronger than in UC.(14) A large part of genetic research and new developments concern CD. However, genetic factors alone are not sufficient to cause CD; after all, concordance in monozygotic twins is only 50%.(14) It is very important to identify the genes playing a role in IBD, because both understanding of aetiology and pathogenesis and know-how on diagnostics and response on therapy will be improved.

In concordance with the multi-factorial character of IBD there are multiple phenotypes. Amongst others, phenotypic diversity is expressed in time of first presentation of disease, disease localization and disease severity.

Polymorphisms and an increased susceptibility for CD

Genome screening yielded several susceptibility loci by now. The first gene identified that is clearly associated with the risk of development of CD is the 'caspase-activation recruitment' domain (CARD15)/ the 'nucleotide-binding' domain (NOD2) gene.(15-17) The NOD2/CARD15 gene is located on chromosome 16. Mutations of the NOD2/CARD15 gene are associated with ileal disease activity, the presence of disease at a younger age, more frequent formation of granulomas and the occurrence of fistulizing or stenotizing phenotypes.(17-22) Mutations of the NOD2/CARD15 gene can be demonstrated in 10-20% of the Caucasian patients. Homozygous carriage of a NOD2/CARD15 gene mutation does not always lead to the development of CD.(23) As can be foreseen in a complex, multifactorial disorder a mutation of the NOD2/CARD15 gene is neither essential nor sufficient to develop CD.

The NOD2/CARD15 gene encodes for a protein that is expressed in the human being in the cytosol of monocytes, granulocytes and dendritic cells, but also in epithelial cells of the gut.(24) These cells all are important in the process of inflammation in CD.(9) The N-terminal part of the NOD2 protein contains two 'caspase-activation recruitment' domains, a centrally located 'nucleotide-binding' domain and 'leucine-rich repeats' (LRR) on the C-terminal end of the protein. Both CARD regions seem to play a role in the activation of the 'nuclear factor kappa-B' (NF-κB) and in the initiation of apoptosis (programmed cell death). NF-κB is a transcription factor that plays a crucial role in the expression of inflammatory cytokines in CD.(9,25) Glucocorticoids can inhibit NF-κB. This mechanism possibly explains the positive effect of steroid treatment in CD. Before NF-κB can be activated, the binding of bacterial components (such as lipopolysaccharide) via LRR is essential.

Three polymorphisms in the NOD2/CARD15 gene are positively associated with an increased risk of susceptibility for CD. It concerns two 'single nucleotide polymorphisms' (SNPs), causing a change of amino acid (Arg702Trp or R702W and Gly908Arg or G908R) and a 'frame shift' mutation with the insertion of the nucleotide cytosine (Leu1007fsinsC or 3020Cins), by which the gene cannot be encoded properly anymore and by which the protein is strongly shortened. The three polymorphisms are all located in the LRR, that are involved in the recognition of pathogens.(15-17,26)

The LRR jointly are a structure in a protein, that can function as a receptor for microbial elements.(27) In the case of the NOD2 protein this element is a protein, the muramyl dipeptide, that is found in the bacterial cell wall.(28) NOD2 is a part of a protein family that occurs in many organisms. In plants these proteins often are involved in resistance against pathogens. In human beings the protein is considered to play a role in the innate immunity. This part of the immune system is not life long 'programmed', in contrast to the adaptive humoral and cellular immunity. It concerns evolutionary, very ancient receptors for pathogens, that, in higher organisms, play a role in the first recognition of microbial invasion.(27)

In vitro studies on functional consequences of the three mutations in the gene have shown that they cause a diminished capacity to activate NF-κB.(17,29) Another argument to the 'loss of function hypothesis' is the observation that humans with homozygous expression of the fore mentioned mutations have a tenfold higher risk than humans with a heterozygous expression.(15,17,21,29)

As the NOD2/CARD15 proteins, Toll-like receptors (TLR's) are pattern recognition receptors that signal presence of bacterial antigens, and play a key role in innate immunity system. NOD2 is located intracellular, whereas TLR's are extra cellular proteins. Both NOD2 and the TLR's are activated by pathogen-associated molecular patterns (PAMP) such as endotoxins.

TLR's are usually membrane bound. These molecules are present on epithelial cells and involved in the first phase of host-response to micro-organisms. Each of these receptors (TLR 1-10) recognizes another molecule of a specific group of micro-organisms.

However, the question remains how a defect in a pro-inflammatory cascade of signals can lead to an inflammatory condition such as CD.

Relevance for clinical practice

The discovery of the NOD2/CARD15-gene caused acceleration in the clarification of the aetiology of CD. By now other loci for vulnerability of CD are examined. Loci are found on chromosome 5, 6, 12 and 14. Next to the current knowledge already acquired with the discovery of abovementioned SNPs in the NOD2/CARD15-gene, it is likely that in the near future more clarity will arise concerning the aetiology of the disease as soon as the genes concerned will be identified on these chromosomes.

Up till now, these findings are of little importance for clinical practice. In The Netherlands 7.4 % of the adult Crohn patients are homozygote or 'compound heterozygote' for the SNPs in the NOD2/CARD15-gene.(30) The risk that comes along with homozygous expression is calculated on a relative risk of 23 with a confidence interval of 6 tot 84.(19) This risk is comparable to the risk that arises as one has a first grade family member with CD (relative risk = 15).(29) Therefore, it seems too early for genetic counselling. It should be noted that in calculating the risk of homozygous expression relatively small control groups, in which no homozygous expression was found, were used. It can be expected that the calculation of the relative risk will be higher if larger control groups are used. If in the near future this risk can be calculated more precisely it is imaginable that a parent suffering from severe CD wants to involve the NOD2/CARD15-genotype of oneself and the life partner in the decision to get pregnant. Genotyping also could be useful in characterizing the phenotype of the disease more precisely; in patients with IC the NOD2/CARD15-genotype could be decisive in the final diagnosis.

The recent understandings in molecular mechanisms concerning the aetiology of inflammatory bowel diseases are of crucial importance for the future therapies of these diseases. Analysis of molecular and cellular processes in normal and disordered immune response has clarified the biological function of cells that are involved in the resistance of the gut. Besides, important knowledge on inflammation is gathered. At the moment this knowledge is used to develop specific therapies. Immunobiologic remedies, such as antibodies against TNF-α and interleukine-12 and antisense oligonucleotides against specific subunits of NF-κB are already being tested in clinical trials. This development has only just started and will increase in interest the next years.

Genetic susceptibility may have a more important role in the aetiology and severity of early-, than of late-onset IBD. If so, a higher frequency of the gene mutations can be expected in paediatric IBD patients. Besides, since phenotype of paediatric IBD differs from adult-onset IBD, genotype-phenotype associations also may differ. Therefore, it is important to perform genetic studies in paediatric IBD populations.

Investigation and diagnosis of IBD

The diagnosis and classification of IBD is based on the clinical presentation, endoscopy, radiology and histology. Distinction between CD and UC is important as prognosis, clinical course and treatment options vary. Besides distinction of the type of inflammatory disease, assessment of disease severity is important to identify patients with active inflammation so that

optimal therapy may be prescribed.(31) Current diagnostic standard of care in paediatric patients suspected of IBD consists of ileocolonoscopy and gastroduodenoscopy with tissue sampling for histological analysis and barium enteroclysis.(32)

Endoscopy in paediatric IBD

Colonoscopy is very important in diagnosing IBD. It enables to inspect the gut mucosa macroscopically and to take mucosal biopsies for histo-pathology. It should always be attempted to reach the terminal ileum, since 10% of paediatric Crohn patients present with isolated terminal ileitis.(1) Moreover, in case of a pancolitis, presence of terminal ileitis may be suggestive for CD instead of UC. It is advised to perform a gastroduodenoscopy in the same diagnostic session, since 10-30% of paediatric-onset CD can only be diagnosed due to histological changes in the upper gastrointestinal tract.(33-35) Since endoscopy is of high importance in diagnosing IBD it is important to evaluate endoscopic strategies and diagnostic yield in children, suspected of IBD.

Radiology in paediatric IBD

Small bowel barium enteroclysis or barium meal follow through is currently considered the most sensitive technique for imaging of the small bowel and will be able to detect inflammation, stenotizing and/or internal fistulizing. However, radiation exposure and patient discomfort are clear disadvantages, especially in children and adolescents suffering from a chronic disease such as IBD. In their future, these patients are potentially exposed to much more of these investigations.

Therefore, Magnetic Resonance Imaging (MRI) maybe a better alternative. Another advantage of MRI as against enteroclysis is the non-invasive transmural assessment of the bowel, thus theoretically facilitating both the diagnosis of IBD and differentiation between CD and UC. In the past poor contrast resolution and motion artefact precluded the use of MRI for bowel imaging. Technological advances, including the use of intravenous gadolinium, fat suppression and respiration-suspended sequences have extended the role of MRI in the evaluation of the gastrointestinal tract.(36-39)

Although a distinction between CD and UC can often be made based on well-established clinical, endoscopic and histological criteria, in 5-23% of cases differentiation between these two entities is not possible, particularly when disease is limited to the colon.(40) MRI may be a very valuable tool in both diagnosing IBD and follow-up. However, to perform and interpret MRI in children and adolescents with IBD optimally more experience and well-performed studies are needed.

Therapy in paediatric IBD

Little published evidence on treatment in paediatric IBD is available. A lot of paediatric practice comes from studies in adult IBD populations, small paediatric populations or expert opinion. Conventional remission induction consists of either sustained enteral nutrition, 5-aminosalicylic acid or high-dose corticosteroids for a minimum of three weeks. Conventional maintenance

therapy usually consists of azathioprine or methotrexate for a minimum of 4 months. Further details on treatment of IBD are thoroughly reviewed by Escher et al.(41)

Azathioprine in IBD

In CD azathioprine and 6-mercaptopurine are drugs of first choice for maintenance therapy.(42) Especially the steroid sparing effect of these drugs is important in the treatment of children.(43) However, because of the toxicity these drugs are prescribed with caution. Azathioprine is the pro-drug of 6-mercaptopurine, which is metabolized into the active substance 6-thioguanine (6-TG) via some enzymatic conversions. Clinical response on therapy correlates with the level of this metabolite.(44,45) The enzyme thiopurine-methyltransferase (TPMT) plays a role in this conversion and determines the final levels of 6-TG and the hepatotoxic metabolite 6-methyl-mercaptopurine (6-MMP). To high a level of the active 6-TG causes bone marrow suppression, mainly expressed in leucopoenia.

Three decades ago, in a population study, it was shown that the activity of the TPMT enzyme was inherited autosomally co-dominant, in which distinction could be made between individuals with low TPMT activity (phenotype $TPMT^{L}$,1 in 300 individuals), intermediate activity (phenotype $TPMT^{L}$/ $TPMT^{H}$, 11% of the population) and individuals with a normal activity (the $TPMT^{H}$/ $TPMT^{H}$ phenotype).(46) The different alleles, which are at the basis of these phenotypes, are described by now. The variant allele TPMT*3A is the most frequent prevailing mutation in Caucasian populations and contains amongst others the polymorphisms Ala154Thr and Tyr240Lys.(47) Homozygotes have the $TPMT^{L}$ phenotype, but other polymorphisms, with a varying prevalence in the different populations are described.(48,49)

The phenotype of the TPMT gene is important for the height of the 6-TG levels. The 6-TG levels determine the effectiveness of the therapy and the chance of bone marrow suppression. In a group of 92 children of IBD the heterozygotes for the TPMT-gene appeared to have a significantly higher level of 6-TG.(45) Clinical response on therapy turned out to be linked to 6-TG levels higher than 235 pico-mol per 8.10^{8} red blood cells. The genotyping of patients is not the panacea that can be used for optimizing therapy and prevention of complications. Critics argue that only 1 in 300 individuals is homozygote for mutant alleles of the TPMT-gene and that in patients developing leucopoenia only a minor part is a homozygote or heterozygote mutant (10 and 17%, respectively).(48,50) It is clear that leucopoenia also can occur with normal TPMT alleles.(51) Supporters argue that TPMT-activity is the most important factor influencing the levels of the therapeutic and potentially toxic 6-TG metabolites. They state that knowledge of the TPMT genotype or phenotype should be reflected in the initial dosing of azathioprine and that the potential seriousness of the leucopoenia justifies a screening by which this can be prevented in 1 out of 300 individuals.(51) Presently, it is unclear whether TPMT genotyping would prevent adverse events caused by azathioprine in paediatric IBD patients. The optimal timing of introduction of azathioprine in paediatric CD patients is still subject to debate.

Infliximab in paediatric IBD

Tumour necrosis factor-α (TNF-α) has a key role in the inflammation cascade of CD and is found in increased amount in gut mucosa of Crohn patients.(52) Increased concentrations of TNF-α are found in the faeces of children with CD.(53) A recent development in therapy resistant CD (persistent severe disease activity in spite of abovementioned drugs) is infliximab (IFX). IFX is a monoclonal antibody containing 25% murine epitopes and binds to human TNF-α. Nevertheless, neutralisation of TNF-α is not the main therapeutic effect of IFX. IFX induces apoptosis of lamina propria T-cells. In Crohn patients, these T-cells seem to be resistant to apoptosis. IFX probably binds to activated peripheral lymphocytes and lamina propria T-cells and induces apoptosis via activation of caspase.(54,55)

IFX has become a very potent drug in both adult and paediatric CD patients not responding to standard therapies. However, the safety of infliximab still is of major concern. There are no data on the long-term safety profile of infliximab in the paediatric setting, nor for long-term risks for development of malignancies and auto-immune disorders. It is not clear whether infliximab should be prescribed as maintenance therapy or episodic and when long-term infliximab treatment should or can be discontinued. Early-onset disease may be more susceptible to infliximab. Moreover, infliximab therapy might be disease-modifying in the longer term. Profound knowledge on both efficacy and safety of infliximab therapy in paediatric CD patients is essential. So far, infliximab is started after failure of conventional therapy, consisting of corticosteroids, azathioprine or methotrexate. It might be a wiser approach to start infliximab as first line treatment.

Prognosis of paediatric IBD

IBD is a chronic disease and often has a relapsing course with significant disease morbidity as a consequence. While UC patients can be "cured" by undergoing colectomy, so far CD cannot. Mortality from IBD is extremely rare, morbidity however can be considerable. Growth failure is common. Many patients develop stricturing or fistulizing disease behaviours over time. Having a chronic disease has a high psychosocial impact both on children and adolescents and their families. Both education on IBD and support in coping with IBD must be provided.

Conclusion

Though it is known that IBD is a multi-factorial disorder with a complex interaction between immunological, genetic and bacterial factors, the exact causes of inflammation of the gut are still unknown. Research on susceptibility gene polymorphisms and genotype-phenotype associations may further unravel this aetiology. Since children were less exposed to environmental factors, a higher frequency of gene mutations can be expected in paediatric IBD patients. Learning more on the aetiology is of crucial importance for future therapies of IBD.

The diagnosis and classification of IBD is based on the clinical presentation, endoscopy, radiology and histology. In their future, patients often need repeated endoscopic and radiologic investigations, having clear disadvantages such as patient discomfort and radiation exposure. Therefore it is important to study the optimal diagnostic strategies and the diagnostic yield of these investigations.

The therapeutic azathioprine is widely used as maintenance therapy in paediatric IBD patients. Pharmacogenetic analysis and early introduction of azathioprine might improve both efficacy and safety of this drug. Infliximab is a recent development in the treatment of refractory CD and seems a very potent drug in a population not responding to standard therapies. Evaluation of both efficacy and safety in a patient group dealing with a chronic disease and probably lifelong treatment however is essential.

Outline of the thesis

In this thesis, a series of studies is presented that concern aetiology, diagnostics and treatment of paediatric IBD. Special consideration will be given to genetic influence and infliximab.

Chapter 2 aims to delineate the genetic factors concerning paediatric IBD. The purpose of our study was to determine the frequency of the mutations of the IBD susceptibility genes in paediatric-onset IBD, compare these data with adult-onset IBD and to identify genotype-phenotype associations.

In **Chapter 3** endoscopy used in children with (suspicion of) IBD is further evaluated. For this purpose, 147 pancolonoscopies in children presenting with the chief complaint of rectal bleeding were reviewed.

To investigate the influence of TPMT and ITPase polymorphisms on adverse effects of azathioprine in children with IBD, these polymorphisms and adverse events from azathioprine therapy in a Dutch paediatric IBD population were evaluated in **Chapter 4.**

In **Chapter 5**, the aim was to determine the timing of introduction of azathioprine in newly diagnosed paediatric CD patients over the years 1998-2003, and whether the use of azathioprine was associated with the maintenance of first remission.

Furthermore the aim was to improve knowledge on infliximab therapy in paediatric CD. In **Chapter 6** the current knowledge on biological therapy in paediatric IBD is reviewed. Since top-down therapy might be a wiser approach than step-up in **Chapter 7** a girl with severe Crohn's colitis is presented treated with infliximab and azathioprine as first line treatment. The efficacy and safety of infliximab therapy in children with refractory CD in The Netherlands is evaluated and presented in **Chapter 8** and **9**.

Finally, a summary, a discussion and future perspectives will be given.

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Chapter 2

Differences in genotype and phenotype in paediatric-onset Inflammatory Bowel Disease and adult-onset Inflammatory Bowel Disease

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Abstract

Objective

Genetic susceptibility may have a more important role in the aetiology of early-, than of late-onset IBD. If so, a higher frequency of the gene mutations can be expected in paediatric-onset IBD patients. We aimed to determine frequencies of NOD2/CARD15, TLR4, OCTN and DLG5 mutations in paediatric-onset IBD and compare these data with adult-onset IBD and controls identifying genotype-phenotype associations.

Methods

In total, 103 paediatric-onset and 696 adult-onset IBD patients participated. Phenotypic classification was based on disease localization and behaviour. We assessed the polymorphisms R702W, G908R and 3020insC of NOD2/CARD15; Asp299Gly and Thr399Ile of TLR4; -207G→C, 1672C→T (L503F), rs3792876, rs274551, rs272893 and rs273900 of OCTN; and 113G→A as well as rs2289311, rs1270912 and rs2165047 of DLG5.

Results

Homozygosity for the 3020Cins mutation was significantly higher in paediatric-onset than in adult-onset CD (4.2% v 0.6%, 95% confidence interval (CI) 1.2-42.0). Homozygosity for the OCTN rs3792876 SNP also was significantly higher in paediatric-onset CD than in adult-onset CD (6.1% v 1.1%, p=0.02). The 3020Cins mutation was associated with ileal involvement (1.9% v 13.3%, CI 1.0-53.8) and a positive family history (6.1% v 20%, CI 1.2-9.0). DLG5 rs2165047 was significantly associated with perianal disease (50% v 21.2%, CI 1.4-4).

Conclusions

This study demonstrated that both 3020Cins and OCTN rs3792876 mutations occurred statistically significant more often in paediatric-onset compared to adult-onset CD. Moreover, the 3020Cins mutation in NOD2/CARD15 and DLG5 rs2165047 mutations in this paediatric-onset CD cohort were associated with specific phenotypes.

Introduction

Inflammatory Bowel Diseases (IBD) is a group of disorders marked by chronic idiopathic inflammation of the intestinal tract. IBD comprehends two distinct disease entities, ulcerative colitis (UC) and Crohn's disease (CD). These two groups are defined on clinical and histo-pathological features, but overlapping syndromes occur. As a multi-factorial disorder IBD is caused by a complex interaction of genetic, bacterial and immunological factors. The disease presents before the age of 20 years in 20-25% of patients. The incidence of paediatric IBD (under the age of 18 years) in The Netherlands is recently assessed and concerns 5.2/100.000 children.(1) This is comparable to data from other European countries.(2,3)

Genetic risk factors for IBD have been extensively studied in the last decades. Through fine mapping of genome wide scans and linkage studies the NOD2/CARD15 gene on chromosome 16 has been identified as a susceptibility gene for IBD.(4-6) Three polymorphisms in the CARD15 gene are independently associated with an increased risk of susceptibility for Crohn's disease in Caucasian patients. Two single nucleotide polymorphisms (SNPs) cause a change of amino acids (R702W and G908R) and the third SNP causes a 'frame shift' mutation with the insertion of the nucleotide cytosine (1007fs; 3020Cins), leading to truncation of the NOD2 protein. Mutations of the CARD15 gene are associated with ileal disease activity, the presence of disease at a younger age, more frequent formation of granulomas and the occurrence of penetrating or stricturing phenotypes.(7-11) These can be demonstrated in 5-15% of the Caucasian patients.(12) A high diversity of these mutations exists in different (healthy) European populations. In a mixed European population the three mutations R702W, G908R and 3020Cins are found in 4.0, 1.0 and 1.9%, respectively while in a Dutch population the mutations G908R and 3020Cins are found in 3.0 and 1.0%, respectively.(10,13)

The NOD2/CARD15 proteins and the Toll-like receptors (TLR's) are pattern recognition receptors that signal presence of bacterial antigens, and play a key role in the innate immunity system. NOD2 is located intracellular, whereas TLR's are extracellular proteins. Both NOD2 and the TLR's are activated by pathogen-associated molecular patterns (PAMP) such as endotoxins. The precise mechanism how mutations in the NOD2/CARD15 gene lead to disease susceptibility is unclear. Currently, there are three hypotheses. According to the first hypothe-sis intact NOD2 signalling inhibits TLR2-mediated activation of NF-κB. The NOD2 mutations disrupt this putative negative feedback mechanism leading to hyperresponsiveness to luminal bacterial antigens.(14,15) The second hypothesis states that the lack of α-defensin production is the underlying mechanism. Non-mutated NOD2 induces secretion of antimicrobial peptides, known as α-defensins. By contrast, in mucosa in which NOD2 is deficient, the lack of α-defensin production leads to bacterial overgrowth that triggers the inflammatory response of CD.(16) The third hypothesis assumes increased interleukin-1 β synthesis based on experiments with knock-in mice carrying the NOD2 frameshift mutation. These mice showed increased levels of interleukin-1 β in a DSS-induced colitis model. However, these data cannot explain the fact that peripheral blood monocytes of CD patients homozygous for the frameshift mutation show defective interleukin-1 β production.(15)

Recent studies indicate that members of the TLR family may also increase IBD susceptibility.(17) The Asp299Gly polymorphism in TLR4 was associated with CD and UC.(18) Another TLR4 mutation, Thr399Ile was associated with UC.(19)

Of late, two novel IBD susceptibility genes have been identified, being the carnitine / organic cation transporter (OCTN) and Drosophila Discs Large Homologue 5 (DLG5). Within the IBD5 locus, a 250 kb risk haplotype on chromosome 5q31 has been associated with CD in different cohorts (20-22). However, the causative gene has not been clearly recognized due to a high degree of linkage disequilibrium in this region. Peltekova et al. identified two functionally relevant mutations in OCTN genes on the IBD5 locus being responsible for the IBD5 association. These mutations concern variants of SLC22A4 and SLC22A5 encoding OCTN1 and OCTN2.(23) The T substitution of C1672 in exon 9 of the SLC22A4 gene encodes for OCTN1 and a G to C substitution at position -207 in the promoter region of SLC22A5 gene encodes for OCTN2. Together these polymorphisms form a two allele risk haplotype associated with CD susceptibility. Interaction of this haplotype with mutations in the CARD15 gene is suggested.(24) Other studies could not confirm this association of the two allele risk haplotype of OCTN and CD, though in one study an increased risk in a subgroup with perianal CD has been found.(25,26)

Recently Stoll et al. identified an association between genetic variations in DLG5 on chromosome 10q23 and the risk of developing IBD.(27) DLG5 is important in maintaining the epithelial structure and genetic variants could result in impaired intestinal permeability. Two haplotypes were involved in IBD and CD susceptibility. A SNP 113G→A, resulting in an amino acid substitution R30Q, was positively associated with IBD and CD patients. A second haplotype identified by eight other tagging SNPs was protective for IBD. Gene-gene interaction with CARD15 in CD was detected by a significant difference in association of the 113A variant in patients carrying the risk alleles for CARD15 compared to patients not carrying these alleles. However several subsequent studies failed to show any association for DLG5 with IBD.(28-32)

Genetic susceptibility may have a more important role in the aetiology of early-, than of late-onset IBD. After all, early-onset IBD patients were less exposed to environmental factors than late-onset IBD patients. If so, a higher frequency of the gene mutations can be expected in paediatric IBD patients. The purpose of our study was to determine the frequency of NOD2/CARD15, TLR4, OCTN and DLG5 mutations in paediatric-onset IBD, compare these data with adult-onset IBD and to identify genotype-phenotype associations.

Materials and Methods

Study patients

From November 1st 2003 to February 1st 2006, 103 paediatric-onset (under the age of 19) IBD patients agreed to participate in this genetic study performed in the Emma Children's

Hospital/Academic Medical Centre. From January 1st 1997 to April 1st 2005, in the Academic Medical Centre, 696 adult-onset (from the age of 19) IBD patients agreed to participate in this study. IBD was diagnosed based on clinical, endoscopic, radiological and histological criteria of Lennard-Jones.(33) Patients with indeterminate colitis were excluded. Of all patients, written informed consent was obtained, and the study protocol was approved by the institutional review board of the Emma Children's Hospital/Academic Medical Centre, Amsterdam. Haplotypes of spouses of IBD patients from the University Medical Center Groningen were taken as controls. Most of them were already used for genotype-phenotype studies on CARD15 and TLR4.(17,34)

Phenotype analysis

Gender and age at diagnosis of all patients were assessed. Phenotypic classification was based on disease localization and behaviour. Localization was determined by endoscopic, histological and/or radiological examination. Categories were made according to the Vienna classification: upper gastrointestinal tract (including jejunum or upper ileum), ileal, ileo-colonic (terminal ileum or terminal ileum and ascending colon), colonic (transverse colon, descending colon, sigmoid or rectum) and perianal disease. Behaviour was defined as uncomplicated (non-stricturing/non-penetrating), penetrating or stricturing. Extraintestinal manifestations included eye, joint, skin and liver involvement. Surgical intervention was defined as any operative IBD-related procedure, such as gut resection or fistula correction. Family history was defined as positive if at least one first- or second-degree relative was diagnosed with IBD. Phenotypic assessment was mostly available by the IBD-database and completed by the treating paediatric gastroenterologists, the treating gastroenterologists and by chart review.

Genotype analysis

Venous blood samples (10 ml from each paediatric patient and 20 ml from each adult patient) were collected. DNA was extracted following standard procedures and was stored at -80 °C. Primers to amplify the polymorphic loci were selected using on-line Primer3 software. SNP genotyping was carried out by using TaqMan PCR primer/probe sets, designed through Applied Biosystems' Assay by Design service (http://myscience.appliedbiosystems.com/, Foster City, USA). SNP assay reactions were performed in 5 µl volumes and contained 25 ng DNA, 1x TaqMan Universal PCR Master Mix (Applied Biosystems), 100 nM of each primer and 900 nM of each probe. Cycling conditions on the ABI prism 7900 HT (Applied Biosystems) were 2 minutes 50°C, 10 minutes 95°C followed by 40 cycles of 15 seconds 92°C and 1 minute 60°C. End-point fluorescence was measured immediately after cycling. Alleles were assigned using SDS 2.0 software (Applied Biosystems).

DNA of the patients was screened for the R702W, G908R and 3020Cins polymorphisms of NOD2/CARD15 and the Asp299Gly and Thr399Ile polymorphisms of TLR4. For OCTN the known polymorphisms -207G→C and 1672C→T (L503F) and four additional SNPs (rs3792876, rs274551, rs272893 and rs273900) were analyzed. These SNPs were selected as haplotype-tagging SNPs for the most common haplotypes covering the entire SLC22A4

/ SLC22A5 gene region. For DLG5, the previously described 113G→A polymorphism was determined. SNP rs2289311 was used as a haplotype-tagging SNP for the undertransmitted DLG5_26 haplotype described by Stoll et al.(19) Three added SNPs (rs1270912, rs228931 and rs2165047) were selected as haplotype-tagging SNPs to cover DLG5 based on information from the HAPMAP database (http://www.hapmap.org).

Correlations

Genotype correlations between paediatric-onset IBD, adult-onset IBD and healthy control populations were examined. Phenotype correlations in paediatric-onset and adult-onset IBD population were examined. Consequently, genotype-phenotype correlations in paediatric-onset IBD were examined and compared to genotype-phenotype correlations in adult-onset IBD.

Statistics

The differences between the frequencies of the NOD2/CARD15, TLR4, OCTN and DLG5 polymorphisms in paediatric-onset patients were compared with adult-onset patients and healthy controls using χ^2, when valid, otherwise using Fisher's exact tests. Genotypic association analysis of CD characteristics was also performed in subsets of CD patients stratified according to the Vienna classification and comparing them with controls. Gene-gene interactions (NOD2/CARD15 interaction with TLR4, OCTN and DLG5) were tested by means of logistic regression in SPSS. A p-value of <0.05 was considered to be significant.

Results

Phenotypes

A total of 103 paediatric-onset IBD patients and 696 adult-onset IBD patients were included. Patient characteristics are described in Table 1a and 1b.

Isolated ileal disease is more common in adult-onset CD when compared to paediatric-onset CD (p=0.0004). Paediatric-onset UC more often is a pancolitis when compared to adult disease (p<0.0001). Adult-onset CD patients more often have stricturing and/or penetrating disease behaviour and more frequently undergo surgery in comparison to paediatric-onset CD patients.

Association analyses

Genotype and allele frequencies for the NOD2/CARD15 polymorphisms R702W, G908R and 3020Cins are detailed in table 2a.

A statistical association for R702W was found with paediatric-onset CD, carriership in 14 patients with CD 14 [19.5%] and in patients with paediatric-onset UC, 7 [23.3%] versus controls 17 [6.3%]: CD p=0.0027, UC p=0.0063. For the 3020Cins SNP, a nearly statistical association was found with paediatric-onset CD (carriership of 3020Cins in patients with CD

Table 1a. IBD Patient Characteristics

	Paediatric-onset IBD (n=103)	**Adult-onset IBD (n=612)**
Gender (male; female), n (%)	50; 53 (49; 51)	263; 349 (43.0; 57.0)
Age at diagnosis, mean (range in years)	12.0 (0.5-18)	35.3 (19-69)
Type of IBD (CD; UC), n (%)	72; 31 (69.9; 30.1)	386; 226 (63.1; 36.9)
Ethnicity (caucasian; negroid; asiatic), n (%)	88; 9; 6 (85.4; 8.7; 5.8)	NA
Family history of IBD, n (%)	18 (17.5)	NA
Localization UC (proctitis, left-sided, pancolitis), n (%)	6; 6; 19 (19.4; 19.4; 61.3) #	60; 112; 54 (26.5; 49.6; 23.9)

Table 1b. CD Patient Characteristics

	Paediatric-onset CD (n=72)	**Adult-onset CD (n=368)**
Gender (male; female), n (%)	40; 32 (56; 44)	134; 252 (35; 65)
Age at diagnosis, mean (range in years)	14 (6-18)	42 (19-70)
Localization (ileal; colonic; ileo-colonic), n (%)	16; 26; 29 (22.2; 36.1; 40.3)$	153; 100; 76; (41.6; 25.9; 19.7)
Localization upper; peri-anal, n (%)	NA; 13 (NA; 18)	176; NA (45.6; NA)
Disease behaviour (nonstricturing/ nonpenetrating; structuring; penetrating), n (%)	51, 10, 14 (70.8; 13.9; 19.4)	176; 81; 129 (45.6; 21.0; 33.4)
Extraintestinal manifestations, n (%)	14 (19.4)	97 (25.1)
Operated patients, n (%)	17 (23.6)	173 (44.8)

# p<0.0001

$ p=0.0004

10 [13.9%] versus controls 20 [7.3%]: p=0.060). Homozygosity for the 3020Cins mutation was noted in 3 patients (4.2%) of the paediatric-onset CD cohort, whereas 2 out of 343 patients (0.6%) of the adult-onset CD patients were homozygous (p=0.04, relative risk [RR] 7.1 95% confidence interval [CI] 1.2-42.0). The prevalence of NOD2/CARD15 mutant homozygotes and compound heterozygotes was higher in the paediatric cohort compared to adult-onset CD, but this did not reach statistical significance: 8 out of 72 (11.1%) paediatric-onset CD patients and 21 out of 293 adult-onset CD patients (7.2%) were either compound heterozygous or homozygous for NOD2/CARD15 mutations. It was however significantly increased compared to controls (8 [2.9%], p=0.005). The polymorphism R702W was significantly increased in adult-onset CD patients compared to controls (p=0.006). The prevalence of NOD2/CARD15 mutant homozygotes and compound heterozygotes also was increased in adult-onset CD patients versus controls (p=0.006, RR1.6 95% CI 1.2-2.0).

For the Asp299Gly and Thr399Ile polymorphisms of TLR4 no significant associations were found between paediatric-onset IBD, CD or UC versus controls or versus adult-onset IBD, CD or UC patients (table 2b).

However, both polymorphisms Asp299Gly and Thr399Ile were significantly increased in adult-onset CD patients compared to controls (p=0.02 and p=0.01, respectively). The prevalence of

Table 2a. Genotype and allele frequencies of NOD2/CARD15 mutations in paediatric-onset IBD patients, adult-onset CD patients and controls

	Paediatric-onset IBD	Paediatric-onset CD	Paediatric-onset UC	Adult-onset CD	Controls
R702W					
Genotype, n (%)					
Wild-type	81 (79.4%)	58 (80.6%)	23 (76.7%)	289 (82.3%)	254 (93.4%)
Homozygous	2 (2.0%)	2 (2.8%)	0 (0.0%)	7 (2.1%)	1 (0.4%)
Heterozygous	19 (18.6%)	12 (16.7%)	7 (23.3%)	51 (15.5%)	17 (6.3%)
p-value (v controls)	0.0003	0.003	0.006		
p-value (v adult-onset)		NS			
p-value (adult-onset v controls)				0.0006	
Mutant allele, n (%)	23 (11.3%)	16 (22.2%)	7 (11.7%)	65 (9.4%)	19 (3.5%)
G908R					
Genotype, n (%)					
Wild-type	97 (94.2%)	66 (91.7%)	31 (100%)	289 (90.0%)	256 (94.1%)
Homozygous	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (0.9%)	2 (0.7%)
Heterozygous	6 (5.8%)	6 (8.3%)	0 (0.0%)	29 (9.0%)	18 (6.5%)
p-value (v controls)	NS	NS	NS		
p-value (v adult-onset)		NS			
p-value (adult-onset v controls)				NS	
Mutant allele, n (%)	6 (2.9%)	6 (4.2%)	0 (0.0%)	35 (5.5%)	22 (4.0%)
3020Cins					
Genotype, n (%)					
Wild-type	91 (88.3%)	62 (86.1)	29 (93.5%)	302 (88.0%)	252 (92.6%)
Homozygous	3 (2.9%)	3 (4.2%)	0 (0.0%)	2 (0.6%)	2 (0.7%)
Heterozygous	9 (8.7%)	7 (9.7%)	2 (6.5%)	39 (11.4%)	18 (6.6%)
p-value (v controls)	NS	NS	NS		
p-value (v adult-onset)		NS			
p-value (adult-onset v controls)				NS	
Mutant allele, n (%)	15 (7.3%)	13 (9.0%)	2 (3.2%)	43 (6.3%)	22 (4.0%)
NOD2					
Compound heterozygous and/or homozygous	9 (8.7%)	8 (11.1%)	1 (3.2%)	21 (7.2%)	8 (2.9%)
p-value (vs controls)	0.017	0.005	NS		
p-value (vs adult-onset)		NS			
p-value (adult-onset v controls)				0.006	

TLR4 mutant homozygotes and compound heterozygotes also was increased in adult-onset CD patients versus controls (p=0.02; RR 1.28; 95% CI 1.1-1.5). The NOD2/CARD15 and TLR Asp299Gly data of the adult-onset IBD patients partially overlap with the cohort analysed and described by Braat et al.(35)

For the OCTN rs3792876 SNP a statistical association was found with paediatric-onset CD (p=0.009 versus controls and p=0.03 versus adult-onset CD) (table 2c).

Table 2b. Genotype and allele frequencies of TLR4 mutations in paediatric-onset IBD patients, adult-onset IBD patients and controls

	Paediatric-onset IBD	Paediatric-onset CD	Paediatric-onset UC	Adult-onset IBD	Adult-onset CD	Adult-onset UC	Controls
Asp299Gly							
Genotype, n (%)							
Wild-type	89 (86.4%)	62 (86.1%)	27 (87.1%)	515 (85.3%)	320 (84.7%)	195 (86.3%)	224 (91.8%)
Homozygous	1 (1.0%)	1 (1.4%)	0 (0.0%)	7 (1.2%)	5 (1.3%)	2 (0.9%)	0 (0.0%)
Heterozygous	13 (12.6%)	9 (12.5%)	4 (12.9%)	82 (13.6%)	53 (14.0%)	29 (12.8%)	20 (8.2%)
p-value (v controls)	NS	NS	NS				
p-value (v adult-onset)	NS	NS	NS				
p-value (adult-onset v controls)				0.02	0.02	NS	
Mutant allele, n (%)	15 (7.3%)	11 (7.6%)	4 (6.5%)	96 (7.9%)	63 (8.3%)	33 (7.3%)	20 (4.1%)
Thr399Ile							
Genotype, n (%)							
Wild-type	90 (87.4%)	63 (87.5%)	27 (87.1%)	486 (84.5%)	298 (83.2%)	188 (86.6%)	224 (91.1%)
Homozygous	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (0.7%)	3 (0.8%)	1 (0.5%)	0 (0.0%)
Heterozygous	13 (12.6%)	9 (12.5%)	4 (12.9%)	85 (15.2%)	57 (15.9%)	28 (12.9%)	22 (8.9%)
p-value (v controls)	NS	NS	NS				
p-value (v adult-onset)	NS	NS	NS				
p-value (adult-onset v controls)				0.03	0.01	NS	
Mutant allele, n (%)	13 (6.3%)	9 (6.3%)	4 (6.5%)	93 (8.3%)	63 (8.8%)	30 (6.9%)	22 (4.5%)
TLR4							
Compound heterozygous and/or homozygous	13 (12.6%)	9 (12.5%)	3 (9.7%)	73 (13.1%)	47 (13.7%)	26 (12.6%)	18 (7.4%)
p-value (v controls)	NS	NS	NS				
p-value (v adult-onset)	NS	NS	NS				
p-value (CD adult-onset v controls)				0.02	0.02	NS	

Homozygosity for the OCTN rs3792876 SNP also appeared significantly increased in paediatric-onset CD patients when compared to the adult-onset CD patients (4 [6.1%] versus 4 [1.1%]; p=0.02). No association with OCTN was found for the two previously described SNPs L503F and -207G→C and three other tagging SNPs with IBD, CD or UC. The OCTN rs272893 and rs273900 SNPs were significantly increased in adult-onset CD patients compared to controls (p=0.04). The OCTN rs274551 SNP was significantly decreased in adult-onset CD patients compared to controls (p=0.002). Ninety-three paediatric-onset IBD patients were genotyped for the DLG5 polymorphisms (table 2d).

Carriership of mutations was equally divided between men and women. For DLG5, rs2165047 tended to be differently distributed among paediatric-onset UC compared to controls, although this was not statistically significant (p=0.088). No associations were found versus the adult-onset IBD population. In adult-onset CD and UC however, the different distribution of DLG5 rs2165047 did reach statistical significance compared to controls (p=0.04 and p=0.03 respectively).

Table 2c. Genotype and allele frequencies of OCTN mutations in paediatric-onset IBD patients, adult-onset IBD patients and controls

	Paediatric-onset IBD	Paediatric-onset CD	Paediatric-onset UC	Adult-onset IBD	Adult-onset CD	Adult-onset UC	Controls
OCTN; rs3792876							
Genotype, n (%)							
Wild-type	79 (85.0%)	54 (81.9%)	24 (96.0%)	570 (83.2%)	315 (84.4%)	255 (83.9%)	248 (86.7%)
Homozygous	4 (4.3%)	4 (6.1%)	0 (0.0%)	5 (0.7%)	4 (1.1%)	1 (0.3%)	1 (0.4%)
Heterozygous	10 (10.8%)	8 (12.1%)	1 (4.0%)	110 (16.1%)	62 (14.5%)	48 (15.8%)	37 (12.9%)
p-value (v controls)	0.03	0.009	NS				
p-value (v adult-onset)	0.01	0,03	NS				
p-value (adult-onset v controls)				NS	NS	NS	
Mutant allele, n (%)	18 (9.7%)	16 (12.1%)	1 (2.0%)	112 (8.2%)	62 (8.3%)	50 (8.2%)	39 (6.8%)
p-value (v controls)	NS	0.04	NS				
OCTN; rs272893							
Genotype, n (%)							
Wild-type	44 (47.8%)	28 (43.8%)	14 (53.8%)	274 (40.5%)	158 (42.5%)	116 (38.0%)	124 (42.6%)
Homozygous	10 (10.9%)	8 (12.5%)	2 (7.7%)	92 (13.6%)	55 (14.8%)	37 (12.1%)	25 (8.8%)
Heterozygous	38 (41.3%)	28 (43.8%)	10 (38.5%)	311 (45.9%)	159 (42.7%)	152 (49.8%)	141 (48.6%)
p-value (v controls)	NS	NS	NS				
p-value (v adult-onset)	NS	NS	NS				
p-value (adult-onset v controls)				NS	0.04	NS	
Mutant allele, n (%)	58 (31.5%)	44 (34.3%)	14 (26.9%)	495 (36.6%)	269 (36.2%)	226 (37.0%)	191 (32.9%)
OCTN: rs1050152							
Genotype, n (%)							
Wild-type	31 (33.7%)	24 (36.9%)	7 (28.0%)	225 (33.1%)	117 (31.1%)	108 (35.5%)	88 (30.0 %)
Homozygous	19 (20.7%)	14 (21.5%)	4 (16.0%)	135 (19.9%)	76 (20.2%)	59 (19.4%)	55 (18.8%)
Heterozygous	42 (45.7%)	38 (41.5%)	14 (56.0%)	320 (47.1%)	183 (48.7%)	137 (45.1%)	150 (51.2%)
p-value (v controls)	NS	NS	NS				
p-value (v adult-onset)	NS	NS	NS				
p-value (adult-onset v controls)				NS	NS	NS	
Mutant allele, n (%)	80 (43.5%)	66 (50.8%)	22 (44%)	590 (43.4%)	335 (44.5%)	255 (41.9%)	260 (44.5%)
OCTN; rs273900							
Genotype, n (%)							
Wild-type	36 (40.4%)	23 (37.1%)	12 (46.2%)	269 (38.6%)	154 (39.4%)	115 (37.7%)	125 (43.1%)
Homozygous	10 (11.2%)	8 (12.9%)	2 (7.7%)	100 (14.4%)	63 (16.1%)	37 (12.1%)	24 (8.3%)
Heterozygous	43 (48.3%)	31 (50.0%)	12 (46.2)%	327 (47.0%)	174 (44.5%)	153 (50.2%)	141 (48.6%)
p-value (v controls)	NS	NS	NS				
p-value (v adult-onset)	NS	NS	NS				
p-value (adult-onset v controls)				NS	0.01	NS	
Mutant allele, n (%)	63 (35.4%)	47 (37.9%)	16 (30.8%)	527 (75.8%)	300 (38.4%)	227 (37.2%)	189 (32.6%)

	Paediatric-onset IBD	Paediatric-onset CD	Paediatric-onset UC	Adult-onset IBD	Adult-onset CD	Adult-onset UC	Controls
OCTN; rs274551							
Genotype, n (%)							
Wild-type	58 (63.0%)	43 (65.2%)	14 (56.0%)	481 (70.8%)	276 (74.0%)	205 (67.0%)	187 (64.8%)
Homozygous	5 (5.4%)	4 (6.1%)	1 (4.0%)	17 (2.5%)	6 (1.6%)	11 (3.6%)	18 (6.3%)
Heterozygous	29 (31.5%)	19 (28.8%)	10 (40.0%)	181 (26.7%)	91 (24.4%)	90 (29.5%)	83 (29.0%)
p-value (v controls)	NS	NS	NS				
p-value (v adult-onset)	NS	NS	NS				
p-value (adult-onset v controls)				0.01	0.002	NS	
Mutant allele, n (%)	39 (21.2%)	27 (20.5%)	12 (24.0%)	215 (15.8%)	103 (13.8%)	112 (18.3%)	119 (20.7%)
OCTN; rs2631367							
Genotype, n (%)							
Wild-type	18 (19.8%)	14 (21.9%)	4 (16.0%)	173 (26.9%)	94 (26.8%)	79 (27.0%)	70 (25.0%)
Homozygous	22 (24.2%)	18 (28.1%)	3 (12.0%)	147 (22.8%)	79 (22.5%)	68 (23.2%)	66 (23.4%)
Heterozygous	51 (56.0%)	32 (50.0%)	18 (72.0%)	324 (50.3%)	178 (50.7%)	146 (49.8%)	145 (51,6%)
p-value (v controls)	NS	NS	NS				
p-value (v adult-onset)	NS	NS	NS				
p-value (adult-onset v controls)				NS	NS	NS	
Mutant allele, n (%)	95 (52.2%)	68 (53.1%)	24 (48.0%)	618 (48.0%)	336 (47.9%)	282 (48.1%)	277 (49.3%)

Gene-gene interactions

No interactions of TLR4, OCTN or DLG5 with CARD15 were detected.

Genotype-phenotype correlations paediatric-onset CD patients

Genotype-phenotype correlations of the paediatric-onset CD patients are shown in table 3. The 3020Cins mutation was associated with ileal involvement (including ileocolonic localization) (p=0.03; RR 7.2; 95% CI 1.0-53.8) and this was even more strongly associated with purely ileal disease (p<0.0001; RR 12.2; 95% CI 4.1-36.4). The 3020Cins mutation was suggestively associated with a positive family history (p=0.07).

DLG5 rs2165047 was significantly associated with perianal disease (p=0.003; RR 2.4; 95% CI 1.4-4.0). Other genotype/phenotype correlations were not found in the paediatric-onset CD cohort.

Discussion

This is the first study to report the contribution of the NOD2/CARD15, TLR4, OCTN and DLG5 genes in a paediatric-onset IBD population and compare these data to an adult-onset IBD population.

Table 2d. Genotype and allele frequencies of DLG5 mutations in paediatric-onset IBD patients, adult-onset IBD patients and controls

	Paediatric-onset IBD	Paediatric-onset CD	Paediatric-onset UC	Adult-onset IBD	Adult-onset CD	Adult-onset UC	Controls
DLG5; rs2165047							
Genotype, n (%)							
Wild-type	45 (48.4%)	35 (53.9%)	9 (34.6%)	331 (49.0%)	181 (48.8%)	150 (49.2%)	161 (55.7%)
Homozygous	8 (8.6%)	6 (9.2%)	2 (7.7%)	67 (9.9%)	35 (9.4%)	32 (10.5%)	14 (4.9%)
Heterozygous	40 (43.1%)	24 (36.9%)	15 (57.7%)	278 (41.1%)	155 (41.8%)	123 (40.3%)	114 (39.4%)
p-value (v controls)	NS	NS	NS				
p-value (v adult-onset)	NS	NS	NS				
p-value (adult-onset v controls)				0.02	0.04	0.03	
Mutant allele, n (%)	56 (30.1%)	36 (27.7%)	19 (21.2%)	412 (30.5%)	225 (30.3%)	187 (30.7%)	142 (24.6%)
DLG5; rs2289311							
Genotype, n (%)							
Wild-type	42 (45.7%)	29 (44.6%)	13 (52.0%)	333 (49.5%)	175 (47.4%)	158 (52.0%)	120 (41.3%)
Homozygous	8 (8.7%)	5 (7.7%)	3 (12.0%)	75 (11.1%)	39 (10.6%)	36 (11.8%)	43 (14.9%)
Heterozygous	42 (45.7%)	31 (47.7%)	9 (36%)	265 (39.4%)	155 (42.0%)	110 (36.2%)	126 (43.8%)
p-value (v controls)	NS	NS	NS				
p-value (v adult-onset)	NS	NS	NS				
p-value (adult-onset v controls)				NS	NS	NS	
Mutant allele, n (%)	58 (31.5%)	41 (31.5%)	15 (30%)	415 (30.8%)	233 (31.6%)	182 (29.9%)	212 (36.7%)
DLG5; rs1270912							
Genotype, n (%)							
Wild-type	43 (46.7%)	31 (47.0%)	11 (44.0%)	316 (46.7%)	174 (46.9%)	142 (46.6%)	133 (45.2%)
Homozygous	12 (13.0%)	8 (12.1%)	4 (16.0%)	84 (12.4%)	44 (11.9%)	40 (13.1%)	31 (10.8%)
Heterozygous	37 (40.2%)	27 (40.9%)	10 (40.0%)	276 (40.8%)	153 (41.2%)	123 (40.3%)	129 (44.0%)
p-value (v controls)	NS	NS	NS				
p-value (v adult-onset)	NS	NS	NS				
p-value (adult-onset v controls)				NS	NS	NS	
Mutant allele, n (%)	61 (33.2%)	43 (32.6%)	18 (36%)	444 (32.8%)	241 (32.5%)	203 (33.3%)	191 (32.6)
DLG5; rs1248696							
Genotype, n (%)							
Wild-type	68 (80.4%)	53 (80.3%)	20 (80.0%)	541 (80.1%)	296 (79.1%)	245 (81.4%)	227 (77.6%)
Homozygous	1 (1.1%)	0 (0.0%)	1 (4.0%)	9 (1.3%)	5 (1.3%)	4 (1.3%)	5 (1.7%)
Heterozygous	17 (18.5%)	13 (19.7%)	4 (16.0%)	125 (18.5%)	73 (19.5%)	52 (17.3%)	61 (20.6%)
p-value (v controls)	NS	NS	NS				
p-value (v adult-onset)	NS	NS	NS				
p-value (adult-onset v controls)				NS	NS	NS	
Mutant allele, n (%)	19 (10.3%)	13 (9.8%)	6 (12%)	143 (10.6%)	83 (11.1%)	60 (10.0%)	71 (12.1%)

Table 3. Mutant allele frequencies in subgroups of paediatric-onset Crohn's Disease

	Number (%), p values included in case of significance						
	R702W	G908R	3020Cins	Asp299Gly	Thr399Ile	OCTN rs3792876	DLG5 rs2165047
Localisation							
Ileum (n=45)	12 (13.3)	4 (4.4)	12 (13.3)	5 (5,6)	5 (5,6)	9 (10.0)	23 (52.3)
Non ileal (n=27)	4 (7.4)	2 (3.7)	1 (1.9)$	4 (7.4)	4 (7.4)	10 (18.5)	19 (35.2)
Behaviour							
Nonstricturing, nonpenetrating (n=51)	15 (14.7)	4 (3.9)	6 (5.9)	6 (5.9)	6 (5.9)	12 (11.8)	24 (23.5)
Stricturing (n=10)	1 (5.0)	1 (5.0)	5 (25.0)#	2 (10.0)	2 (10.0)	4 (20)	4 (20.0)
Penetrating (n=14)	2 (7.1)	1 (3.6)	2 (7.1)	3 (10.7)	3 (10.7)	3 (10.7)	11 (39.3)
Operated (n=17)	2 (5.9)	1 (2.9)	6 (17.6)	6 (17.6)	6 (17.6)	6 (17.6)	11 (32.4)
Not operated (n=55)	14 (12,7)	5 (4.5)	7 (6.4)	12 (10.9)	12 (10.9)	11 (10.0)	30 (27.3)
Perianal disease (n=13)	2 (7.7)	1 (3.8)	2 (7.7)	2 (7.7)	2 (7.7)	5 (19.2)	13 (50.0)*
No perianal disease (n=59)	14 (11.9)	5 (4.2)	11 (9.3)	7 (5.9)	7 (5.9)	12 (10.2)	25 (21.2)
Extraintestinal disease (n=14)	7 (25)	1 (3.6)	1 (3.6)	3 (10.7)	3 (10.7)	3 (10.7)	6 (21.4)
No extraintestinal disease (n=58)	11 (9.5)	5 (4.3)	5 (4.3)	6 (5.2)	6 (5.2)	10 (8.6)	29 (25.0)
Family history of IBD (n=15)	2 (6.7	1 (3.3)	6 (20)§	2 (6.7)	2 (6.7)	5 (16.7)	8 (26.7)
No family history of IBD (n=57)	14 (12.3)	5 (4.4)	7 (6.1)	7 (6.1)	7 (6.1)	10 (8.8)	30 (27.3)

$ p= 0.03 RR 7.2 95.0% CI: 1.0-53.8

p= 0.02 RR 4.1, 95.0% CI: 1.5-11.2 (stricturing versus other behaviour)

* p=0.003 RR 2.4 95.0% CI: 1.4-4.0

§ p=0.07 RR 3.3 95% CI 1.2-9.0

NOD2/CARD15

Homozygosity for the 3020Cins NOD2/CARD15 mutation was significantly more frequent in paediatric-onset CD than in adult-onset CD. This finding confirms our hypothesis that this NOD2/CARD15 mutation predisposes for paediatric-onset CD. So far, reports in literature are conflicting. Ferraris et al. found a higher incidence of the three major NOD2/CARD15 mutations in their Italian paediatric CD cohort compared to Italian adult CD patients (p=0.056).(36) Weiss et al. demonstrated a higher prevalence of G908R mutation in 67 Jewish paediatric CD patients as compared to Jewish adult CD patients, though this did not reach statistical significance (p=0.063).(37) Tomer et al. also studied the three major NOD2/CARD15 mutations in 101 paediatric CD patients but found comparable results to the prevalence reported in adult CD patients.(38) However, their paediatric CD population was not compared to an adult CD population from the same geographic area. Leshinsky-Silver et al. did not find a correlation between age of onset of CD and the three major NOD2/CARD15 mutations in 82 paediatric-onset and 107 adult-onset patients.(39) These conflicting results can be explained by large regional and ethnical differences in genotypes, the broad spectrum of phenotypes within IBD and the relatively low numbers of patients included in these studies.

This study supports a statistical association between R702W and a nearly statistical association between 3020Cins and CD in a Dutch paediatric-onset IBD cohort versus healthy

controls. However, no role was demonstrated for the G908R mutation. R702W was also statistically associated with UC (p=0.0063), while most studies show no association to UC.
The genotype-phenotype analysis revealed a strong association between the 3020Cins mutation and CD localization in the terminal ileum. The association between NOD2/CARD15 mutations and involvement of the ileum is well described, both in adult and paediatric populations.(8,40-42) Furthermore, we found that the 3020Cins mutation is suggestively associated with familial disease. This finding is confirmed by a meta-analysis of 42 studies (mainly adult patients), in which more familial disease in NOD2/CARD15 mutation carriers was demonstrated.(42)

TLR4

No significant associations were found for the TLR4 polymorphisms in this paediatric-onset IBD cohort. However, both Asp299Gly and Thr399Ile polymorphisms were associated with CD in our adult-onset cohort. Moreover, these polymorphisms have been associated with CD and UC.(18,43-45) In other studies, these associations were not confirmed.(46,47) Leshinsky-Silver et al. performed TLR4 mutation analysis in 66 paediatric-onset CD patients and 78 adult-onset CD patients and investigated the correlation of mutations and age of onset. TLR4 mutations were not correlated.(39)

OCTN

In our paediatric-onset CD cohort we cannot confirm the associations of the two variants in SLC22A4 and SLC22A5 that were reported by Peltekova et al. They found that the association with the IBD5 locus is strongest for CD patients diagnosed under 16 years of age and this finding was confirmed in another study.(23,20) Nevertheless, Russell et al. reported that the OCTN1/2 variants do not act independently of variants in the IBD5 locus.(48) In our cohort, although numbers are small, SNP rs3792876 was statistically associated with paediatric-onset CD compared with healthy controls. This mutation was also more frequent in the paediatric-onset CD patients when compared to the adult-onset CD patients. As far as we know, this finding has not been reported before. Taken together, these data support the evidence that the OCTN 1/2 variants are genetic risk factors for CD susceptibility. However, due to the high degree of linkage disequilibrium in the IBD5 locus it remains to be seen whether OCTN1/2 variants are responsible for CD susceptibility or that they are in strong linkage disequilibrium-with other true causative genetic variants.
Moreover, we observed a statistically significant higher frequency of the OCTN rs3792876 mutation in the paediatric-onset CD patients compared to the adult-onset CD patients. As far as we know, this comparison is not reported before.

DLG5

We could not confirm the previously described association of DLG5 and IBD in our paediatric cohort. Interestingly, we did find that rs2165047 was significantly increased in both our adult-onset CD and UC population. Several other studies failed to validate the significance of

DLG5 variants as important determinants in IBD susceptibility in adult-onset IBD.(28,30-32) Friedrichs et al. recently demonstrated that carriership of DLG5 mutations was a susceptibility factor for CD in men but not in women.(49) However, DLG5 mutation carriership was equally divided among both sexes in both our paediatric-onset CD patients and the healthy control group. Associations with DLG5 mutation carriership were not found.

DLG5 rs2165047 mutation carriership in our paediatric CD cohort was significantly associated with perianal disease. The number of patients this association is based on is too small to infer any conclusions as yet. Larger studies are needed.

Unfortunately we don't have data on ethnicity of the adult-onset population. However, it is expected to be the same in the paediatric-onset as in the adult-onset population, as it concerns the same geographic area where the patients come from and referral is based on the same conditions. Calculations were also performed in an exclusively Caucasian paediatric-onset IBD cohort. No other statistical significances were revealed compared with the entire cohort, although some became stronger (data not shown).

In conclusion, this study demonstrated that the R702W mutation in NOD2/CARD15 is associated with CD in a Dutch paediatric-onset IBD cohort. Secondly, for OCTN1, SNP rs3792876, but not the previously described SNPs -207G→C and 1672C→T (L503F), is associated with paediatric-onset CD. Moreover, both 3020Cins and OCTN rs3792876 mutations occurred statistically significant more often in paediatric-onset compared to adult-onset CD. Finally, the 3020Cins mutation in NOD2/CARD15 and DLG5 rs2165047 mutations in this paediatric-onset CD cohort were associated with specific phenotypes. Genetic susceptibility has a more important role in the aetiology of early- than of late-onset CD. Within paediatric-onset CD specific genotype-phenotype associations can be found.

These data stress the importance of genetic susceptibility research in large paediatric-onset IBD cohorts in order to find new genes and to establish the influence of these mutations on disease behaviour.

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Chapter 3

Rectal bleeding in children: Endoscopic evaluation re-visited

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Summary

Objective

Rectal bleeding is an alarming event both for the child and parents. It is hypothesized that colonoscopy instead of sigmoidoscopy and adding esophago-gastro-duodenoscopy in case of accompanying complaints, improves the diagnostic accuracy in children with prolonged rectal bleeding.

Study design

All paediatric patients undergoing colonoscopy because of prolonged rectal bleeding over an 8-year period at the Emma Children's Hospital/Academic Medical Centre were reviewed. Patient demographics, clinical features, number and extent of endoscopic examinations and the endoscopic and histopathological findings were assessed.

Results

A total of 147 colonoscopies were performed in 137 paediatric patients (63 boys) because of prolonged rectal bleeding. Inflammatory bowel disease and polyp(s) were the most prevalent diagnoses. In 72% of patients diagnosed as Crohn's disease, focal, chronically active gastritis was seen on histology, giving support to the diagnosis Crohn's disease. In 22% of the cases polyps would have been missed in case only sigmoidoscopy was performed. No complications after endoscopic intervention were seen.

Conclusions

Colonoscopy is the investigation of choice in children with prolonged rectal bleeding. In patients presenting with accompanying complaints such as abdominal pain or diarrhoea it is advisable to perform ileocolonoscopy combined with esophago-gastro-duodenoscopy. This combines a high diagnostic yield with a safe procedure.

Introduction

Rectal bleeding is an uncommon feature in children. It is an alarming event both for the child and the parents and requires further investigation. The differential diagnosis is extensive and includes anal fissures, hemorrhoids, gastrointestinal infection, inflammatory bowel disease (IBD) and polyps. The first approach to children presenting with rectal bleeding should be inspection and rectal digital examination. However, when gastrointestinal infections and anal fissures due to constipation are ruled out, colonoscopic examination should be performed for diagnosis, prognostic evaluation, and choice of treatment. In The Netherlands, children with an indication for endoscopic examination are referred by general paediatricians to paediatric gastro-enterologists in tertiary centres. Although sigmoidoscopy often is advised in case of rectal bleeding, it is well-known that pathology proximal to the sigmoid can be missed.(1,2,3) Frequently both polyps and inflammation are found higher up in the gastrointestinal tract.(4-8) Therefore colonoscopy should be performed in children presenting with rectal bleeding.(9-11) Today however, colonoscopy is still not a routine procedure. Besides, few recent studies on rectal bleeding in children are available, while morbidity patterns may have changed over time.(1-3)

In our hospital, colonoscopy is performed in children presenting with prolonged rectal bleeding. In case of accompanying complaints such as abdominal pain or diarrhea, IBD might be the cause. In these children, intubation of the terminal ileum is achieved and esophago-gastro-duodenoscopy (EGD) is performed in the same session, according to the Porto criteria (recent ESPGHAN recommendations for diagnosing IBD in children and adolescents).(12) In this guideline upper endoscopy is advocated in all children suspected of IBD, irrespective of presence or absence of upper gastrointestinal symptoms. In all cases biopsies are taken for histopathological examination.

To evaluate the diagnostic yield of this endoscopic policy and to update morbidity patterns we have conducted a retrospective study in children presenting with prolonged rectal bleeding.

Methods

The hospital records of all patients (0-18 years of age) undergoing endoscopy because of prolonged rectal bleeding (minimum duration of one month) at the tertiary centre Emma Children's Hospital/Academic Medical Centre were reviewed, over an 8-year period (January 1998 - November 2005). Both colonoscopies and upper endoscopies were performed under general anesthesia, using Olympus instruments. Either an adult or paediatric type of endoscope was used, depending on age and size of the patient. In our hospital, 3 biopsies are taken routinely from the ileum and subsequently a minimum of two biopsies is taken from every segment of the colon.(12) In case EGD is performed biopsies are taken routinely from the duodenum, anthrum, corpus and esophagus. Retrospectively, patient demographics,

clinical features, number and extent of endoscopic examinations and endoscopic and histopathological findings were evaluated by an independent observer (the second author, A v L).

Results

A total of 144 colonoscopies and 3 sigmoidoscopies were performed in 137 paediatric patients (63 boys) because of prolonged rectal bleeding. Patient demographics and clinical features are shown in table 1. The majority of this population presented with bright red rectal bleeding. Patients usually had longstanding complaints and often presented with accompanying clinical features (table 1). Anaemia (Hb%<10 Gm/dL) was documented in 15 patients (13%) of which 70% were found in IBD patients.

In 80% of patients a clear diagnosis was established after the first colonoscopy. IBD was the most prevalent diagnosis (table 2). All patients but one who were diagnosed IBD presented with abdominal pain and/or diarrhea apart from rectal bleeding. EGD and colonoscopy were performed in 63 patients suspected of IBD, with the terminal ileum being intubated in 62 (98%). In 18 out of 25 patients (72%) diagnosed as Crohn's disease (CD), focal, chronically active gastritis was seen on histology. In all but one patient who also had a Helicobacter pylori infection possibly causing the gastritis, this finding gave support to the diagnosis IBD. Three out of these 18 patients also had granulomas in the upper gastrointestinal tract, confirming the diagnosis of CD. Polyps were the second most prevalent cause in this study cohort and were all found in children under the age of 11 years. All patients diagnosed a polyp reported bright red rectal bleeding but this also accounted 44 out of 46 (96%) patients diagnosed IBD, so the type of bleeding is not helpful in predicting pathology before performing endoscopy. Eight out of the 25 patients (32%) with polyps also presented with abdominal pain while diarrhoea was never reported. In these 25 patients 27 polyps were found. One patient had 3 polyps; all other patients had one polyp. The polyps were located in the rectum (n=11), sigmoid (n=10), descending colon (n=5) or transverse colon (n=1). Therefore, in case a sigmoidoscopy was performed as first procedure, 22% of the polyps would have been missed. Polyps were removed by snare polypectomy and sent for histological examination. All but one were juvenile polyps, while in one patient an inflammatory polyp was found. In

Table 1 Patient demographics and clinical features (n=137)

Gender (male; female)	63; 74
Age at first colonoscopy (mean; range)	9.3 years; 50 days-17.8 years
Ethnicity (Caucasian; Negroid; Asiatic)	111; 15; 11
Accompanying clinical features: (abdominal pain; painful defecation; diarrhoea)	72; 13; 39
Duration rectal bleeding before colonoscopy (mean; range)	28 weeks; 4 weeks-3 years
Type of rectal bleeding (bright red rectal bleeding; blood and clots per rectum; melaena)	127; 5; 5

Table 2 Diagnoses after first and second colonoscopy in children presenting with rectal bleeding (n=137)

Diagnosis	No. of patients (%), first colonoscopy	No. of patients (%), second colonoscopy
Inflammatory bowel disease	46 (33.6%)	3 (30%)
(Crohn's disease; Ulcerative colitis; indeterminate)	(25; 18; 3)	(0; 1; 2)
Polyps	25 (18.2%)	1 (10%)
Constipation with anal fissure or haemorrhoid	9 (6.6%)	1 (10%)
Aspecific proctitis	8 (5.8%)	1 (10%)
Lymphonodular hyperplasia	7 (5.1%)	
Ulcer	3 (2.2%)	
Infectious	1 (0.7%)	1 (10%)
Meckel's diverticulum	1 (0.7%)	
Allergic colitis	1 (0.7%)	1 (10%)
Polyposis coli	1 (0.7%)	
Enterocolitis	1 (0.7%)	
Sarcoidosis	1 (0.7%)	
Amyloidosis	1 (0.7%)	
Eosinophilic colitis	1 (0.7%)	
Coeliac disease	1 (0.7%)	
Haemangioma	1 (0.7%)	
Deviation colitis	1 (0.7 %)	
No abnormalities	28 (20.4%)	2 (20%)

9 patients, an anal fissure or hemorrhoid due to constipation was diagnosed. These patients were all treated with laxatives prior to performing colonoscopy. Since rectal bleeding persisted while constipation was optimally treated, colonoscopy was performed to rule out underlying pathology.

One patient had undergone a Meckel's scan, which was found negative. Because rectal bleeding persisted colonoscopy was done and demonstrated bright red blood coming from the ileum, suggesting a Meckel's diverticulum despite negative scan. The Meckel's scan was revised and found positive after all. In 28 children (20%) presenting with rectal bleeding no abnormalities were found either on colonoscopy or on histopathology. Thirteen out of these 28 patients also presented with abdominal pain and/or diarrhoea. Surprisingly, in one of these patients, familial adenomatous polyposis was diagnosed by genetic analysis, despite negative endoscopy and histopathology. Repeated colonoscopies were performed in 10 patients because of persistent, unexplained complaints. As shown in table 2, this time a cause of rectal bleeding was found in 8 out of 10 patients.

In 140 out of 144 (97%) colonoscopies completion to the coecum was successful. Due to technical problems colonoscopy was not achieved in 4 patients (2 until transverse colon and 2 until descending colon). In 3 patients intentionally sigmoidoscopy instead of colonoscopy was performed: 1) rectal bleeding due to enterocolitis in a boy with Hirschsprung's disease, 2) to rule out other pathology causing rectal bleeding in a patient with constipation and anal

fissures and 3) prolaps of a polyp through the anus which was subsequently removed successfully.
Complications of endoscopy or of anesthesia were not seen.

Discussion

We present a large, retrospective cross-sectional study in children undergoing colonoscopy because of prolonged rectal bleeding. The most common diagnosis in this cohort was IBD. This is in contrast to earlier endoscopic findings (period 1981-1987) both in our hospital and in the literature.(11,13) In that period, polyps were most frequently diagnosed (table 3). This finding confirms the rise in incidence of IBD in childhood.(14) In this study cohort colonoscopy was performed as initial procedure in children presenting with rectal bleeding, combined with intubation of the ileum and EGD in patients also presenting with accompanying complaints such as abdominal pain or diarrhoea. EGD frequently gave support to the diagnosis

Table 3 Historic comparison between causes of rectal bleeding

Period (number of patients)	1981-1987 (n=64)	1998-2005 (n=137)
Normal	34%	20.4%
Anal fissure and/or hemorroid	19%	6.6%
Inflammatory bowel disease	15%	33.6%
Polyps	18%	18.2%

IBD, as described earlier.(15,16) By following this procedure, children diagnosed IBD undergo a thorough work-up in the first diagnostic session according to the present guidelines, stressing the need for performing both colonoscopy with intubation of the terminal ileum and upper endoscopy.(16) Maximal effort to diagnose IBD and determine CD, ulcerative colitis or indeterminate colitis is done and repeated bowel cleansing, sedation or anesthesia because of incomplete work-up is avoided.
The second most common diagnosis in this cohort was polyps. In accordance with earlier studies this study showed that 22% of polyps would have been missed by performing sigmoidoscopy.(5,7,17-19) In these children repeated procedures because of missed polyps also are avoided. These data underline the importance of performing colonoscopy preventing risks missing more proximal polyps. A second risk is missing polyposis coli (defined as presence of more than 5 juvenile polyps in the colon).(20) A third risk is missing a polyp that might undergo malignant transformation, since polyps carry a small but definitive neoplastic potential.(18-21)
Abdominal pain was frequently seen in this study cohort. However, this was even more frequent in the group that was diagnosed as IBD. In case of co-morbidity it is advisable to perform both EGD and ileocolonoscopy.

An important advantage of colonoscopy is the possibility to take biopsies. The importance of histopathology is very well illustrated by the diversity of causes of rectal bleeding, such as sarcoidosis or amyloidosis. Diagnosing celiac disease in one patient probably was coincidental, since rectal bleeding is not a known feature of celiac disease.(22)

In 20% of the children referred for rectal bleeding no abnormalities were found on first colonoscopy. One can hypothesize that rectal bleeding in these patients has been caused by self-limiting diseases such as infection, perianal fissures or hemorrhoids. In case of persistent rectal bleeding further diagnostic work-up such as repeated colonoscopy, Meckel's scan, video capsule endoscopy and double balloon enteroscopy must be considered.(23-26)

Since this is a retrospective study detection bias may have occurred since minor findings on endoscopy may not have been reported and therefore subtle pathology may have been missed. However, in case of persisting complaints colonoscopy was repeated. Since all children presenting with persistent rectal bleeding will be referred to a paediatric gastroenterologist selection bias probably has not occurred. Although this study was not primarily designed to evaluate the success rate of colonoscopy, successful completion was very high (in 97% of patients the coecum was reached, in case of IBD-like symptoms the ileum was intubated in 98% of patients) without any adverse events. Though studies evaluating performance of endoscopy are scarce completion rates vary from 51% to 93%.(27) Our high success rate confirms the efficacy and safety of colonoscopy in children.

Conclusion

Colonoscopy is the investigation of choice in children with prolonged rectal bleeding. In patients presenting with accompanying complaints such as abdominal pain, weight loss and diarrhoea IBD is very likely and therefore ileocolonoscopy combined with EGD should be performed. This combines a high diagnostic yield with a safe procedure.

In case sigmoidoscopy instead of colonoscopy was performed, more than half of the patients would have been incompletely diagnosed. Children diagnosed IBD should undergo ileocolonoscopy and EGD as a second procedure to complete the diagnostic work-up of IBD. One out of 5 polyps would be missed.

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Chapter 4

Pharmacogenetics of thiopurine therapy in paediatric IBD patients

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Summary

Background

Azathioprine (AZA) is widely used in the treatment of children with Inflammatory Bowel Disease (IBD). The occurrence and type of adverse events to AZA may be related to thiopurine S-methyltransferase (TPMT) enzyme activity and to Inosine Triphophate Pyrophosphatase (ITPase) deficiency.

Aim

Investigate frequencies of functional *TPMT* polymorphisms and *ITPA* polymorphisms and their association with the occurrence of adverse events during AZA therapy in a paediatric IBD population.

Methods

Seventy-two AZA treated paediatric IBD patients, 47% girls, mean age 12.5 years (range 6.5-17.5), were assessed for *TPMT* and *ITPA* polymorphisms and for adverse events. The relation between polymorphisms and adverse events is evaluated.

Results

Of all AZA treated patients, 11 experienced an adverse event for which AZA was stopped: pancreatitis (n=4), leucopoenia (n=2) and "general malaise" (n=5). Of the 11 patients who stopped AZA due to adverse events, 10 had wild type alleles for all investigated genotypes. Genotyping of *ITPA 94C>A* polymorphisms showed that two patients were homozygous, both tolerated AZA well.

Conclusions

No association of functional *ITPA* and *TPMT* polymorphisms and the occurrence of AZA related adverse events could be detected. Pharmacogenetic assessment prior to thiopurine therapy seems not warranted.

Introduction

The thiopurine base 6-mercaptopurine (6-MP) and its prodrug azathioprine (AZA) are widely used in the treatment of children with Crohn's disease and ulcerative colitis (Inflammatory Bowel Disease, IBD).(1) The steroid sparing effect of these drugs is of major importance in the treatment of children, since they do not negatively influence growth.(2) A recent study in children with IBD shows that high-dose AZA (3 mg/kg) even may improve growth rate.(3) However, because of its well-known toxicity such as hepatitis, pancreatitis and bone marrow suppression, these drugs are prescribed with caution. AZA is converted into the active substance 6-thioguanine (6-TG) through several enzymatic steps. Levels of 6-TG seem to correlate with clinical response to AZA treatment. However, high 6-TG levels are also associated with increased bone marrow toxicity.(4) The TPMT enzyme plays a role in the inactivation of 6-TG and therefore determines the final levels of 6-TG and the hepatotoxic metabolite 6-methylmercaptopurine (6-MMP). Polymorphisms in the gene encoding the TPMT enzyme are associated with a lower enzyme activity. Heterozygosity for the *TPMT* gene is associated with an intermediate enzyme activity, whereas homozygosity for the mutant alleles is associated with a low or even absent enzyme activity. Thus far, 19 different polymorphisms were found being associated with decreased enzyme activity, of which the *TPMT*1* (wild type allele, 96%) and *TPMT*3* (G460A and/or A719G, 4%) are the most common in Caucasians.(5,6,7) In a group of 92 children with IBD the heterozygotes for *TPMT* gene polymorphisms had a significantly higher level of 6-TG.(8)

Recently, Marinaki et al. identified 2 mutations occurring with polymorphic frequencies that are associated with Inosine Triphophate Pyrophosphatase (ITPase) deficiency, being *94C>A* missense mutation and *IVS2 + 21 A>C* mutation in the *ITPA* gene, which encodes ITPase. The authors predicted an accumulation of the metabolite 6-thio-ITP in ITPase deficient patients treated with thiopurine drugs, resulting in toxicity.(9) ITPase deficiency results in the benign accumulation of the inosine nucleotide ITP. In adults, *94C>A* missense mutation was found to be associated with three adverse drug reactions; being influenza-like symptoms, pancreatitis and rash.(9) *IVS2* mutation was not associated with adverse events.

The aim of this study was to investigate frequencies of *TPMT* polymorphisms and *ITPA* polymorphisms and the association between ITPase deficiency and adverse events from AZA therapy in a Dutch paediatric IBD population.

Materials and Methods

Study patients

From November 1st 2003 to April 1st 2005, in Emma Children's Hospital/Academic Medical Centre and Sophia Children's Hospital/Academic Centre Rotterdam, all paediatric-onset (under the age of 19) IBD patients, in the past or currently treated with AZA were identified

by searching the IBD database and the medical charts. IBD had to be diagnosed based on clinical, endoscopic, radiological and histological criteria of Lennard-Jones.(10) AZA had to be used for a minimum period of 3 months, unless AZA was stopped earlier due to AZA related adverse effects. AZA dosage had to be 2-2.5 mg/kg. Pancreatitis was defined by severe abdominal pain and hyperamylasemia and resolution after withdrawal of AZA; hepatotoxicity by serum alanine transaminases levels greater than twice the upper normal limit (50 IU/l) and resolution after withdrawal of AZA; leucopoenia by a leukocyte count of < 2.5 x 10^9 cells. Influenza-like symptoms included patients with myalgia as a prominent feature. Other complaints such as headache, fatigue or nausea were categorized as "general malaise". These data were extracted from the IBD database, the laboratory database, and the medical charts. Written informed consent was obtained from the patients and their care givers, and the study protocol was approved by the institutional review board.

Genotype analysis

Venous blood samples (10 ml from each paediatric patient) were collected and stored for analysis later on. Genomic screening was accomplished by a PCR and restriction fragment-length polymorphism assay, with specific primers and restriction enzymes for each polymorphism. DNA of the patients was screened for *TPMT*3* (subtype A: both *G460A* and *A719G* mutation, subtype B: *G460A* mutation only, subtype C: *A719G* mutation only), *TPMT*2* (238G>C mutation), *ITPA 94C>A* and *ITPA IVS2 + 21 A>C*.

Statistical analysis

Association analyses were performed using the χ^2 statistics (SPSS, 11.5.2, Chicago, IL, USA). Due to low event numbers in both gene mutations and complications data were pooled. χ^2 Statistics were performed on a cross tab of any mutation in the ITPA or TPMT gene versus any complication due to AZA use.

Results

Patients

A total of 72 patients fulfilled the criteria of the study and were all included. Mean exposure time to AZA in the patient group with adverse events was 9.0 months, in the patient group without adverse events 27.3 months. Patient characteristics are shown in table 1.

Primary outcomes

TPMT and ITPA allele frequencies

Allelic variants of *TPMT* and *ITPA* and their frequencies are shown in table 2.

Homozygosity for *ITPA 94C>A* allele and *ITPA IVS2* alleles has been found in 2 and 1 patient(s), respectively. Two patients were compound heterozygotes for *ITPA 94C>A* allele and *ITPA*

Table 1 Patient Characteristics (n=72)

Gender (male; female)	38; 34
Age at diagnosis (mean; range in years)	12.5; 6.5-17.5
Type of IBD (CD; UC)	57; 15
Ethnicity (Caucasian; Negroid; Asiatic)	60; 9; 3
Exposure time to AZA of patients without adverse events (mean; range in months)	27.3; 4.3-110.0
Exposure time to AZA of patients with adverse events (mean; range in months)	9.0; 1.5-41.0

AZA, azathioprine; IBD, inflammatory bowel disease.

Table 2 Allelic variants of ITPA and TPMT gene and their frequencies among 72 paediatric inflammatory bowel disease patients (n=144 alleles)

allelic variants	n	%	homozygotes/heterozygotes n
TPMT	144	100	72
TPMT*1	139	96.5	0/67
TPMT*2	0	0	0/0
TPMT *3A	3	2.1	0/3
TPMT *3B	0	0	0/0
TPMT *3C	2	1.4	0/2
ITPA	144	100	72
ITPA 94C>A	8	5.6	2/4
ITPA IVS2	13	9.0	1/11

IVS2. Only TPMT *3A and *3C genotypes were identified among all TPMT deficiency associated alleles studied, no patient was homozygote.

Azathioprine related adverse events

In 16 out of 72 patients (22%) AZA was stopped. In 11 patients (15.3%) this was due to adverse events, the other 5 patients (6.9%) had an insufficient response. Adverse events consisted of pancreatitis (n=4), leucopoenia (n=2) and "general malaise" (n=5). None of the patients developed hepatotoxicity or influenza-like symptoms.

Correlation of TPMT and ITPA polymorphism with azathioprine related adverse events

Genotyping of the polymorphisms of *TPMT* and *ITPA* of all patients in association to adverse events are shown in table 3. In the patient group with adverse events, mutations (being heterozygote for both *TPMT*3* and *ITPA IVS2*) were only found in one patient. This patient developed leucopoenia. Genotyping of the *TPMT* polymorphisms revealed that none of the 72 patients was homozygote. Three patients were heterozygote for *TPMT*3A*. All three patients tolerated AZA well, however one patient stopped AZA due to insufficient response. Two patients were heterozygous mutants for *TPMT*3C*. One patient stopped AZA due to insufficient response. The other patient was the one developing leucopoenia. Therefore, AZA was stopped in this patient.

Table 3 polymorphisms of azathioprine treated inflammatory bowel disease patients and adverse effects

		no adverse effects	pancreatitis	leucopoenia	general malaise
TPMT*3	wild type (n=67)	57	4	1	5
	homozygous (n=0)	0	0	0	0
	heterozygous (n=2)	1	0	1	0
	compound heterozygous (n=3)	3	0	0	0
ITPA 94C>A	wild type (n=66)	55	4	2	5
	homozygous (n=2)	2	0	0	0
	heterozygous (n=4)	4	0	0	0
ITPA IVS2	wild type (n=60)	50	4	1	5
	homozygous (n=1)	1	0	0	0
	heterozygous (n=11)	10	0	1	0
ITPA 94C>A and IVS2	compound heterozygous (n=2)	2	0	0	0

Genotyping of *ITPA 94C>A* polymorphisms showed that two patients were homozygote, but AZA was tolerated well in both children. Three of 11 patients with a heterozygous *ITPA IVS2* mutation stopped AZA. Two patients stopped AZA due to insufficient response (after using AZA for 14 weeks respectively 33 months). The other patient developed leucopoenia. Two patients were compound heterozygote for the *ITPA* polymorphisms, and in both children AZA was tolerated well.(Table 3) No statistical significant associations could be detected.

Discussion

AZA was stopped due to toxicity in 15.3% of cases and due to insufficient response in 6.9% of patients. These results of AZA are comparable to other paediatric IBD cohorts.(11) General malaise was found in 6.9%, pancreatitis in 5.6% and leucopoenia in 2.8% of our cohort. This is in accordance with the literature, in which AZA toxicity is reported as general malaise and nausea in 11%, hepatitis in 0.3-1.3%, myelosuppression in 1.4-5.0%, pancreatitis in 1.4-3.3% and immune-mediated symptoms like fever, rash and arthralgia in 2%.(12-15) More importantly this study clearly shows the lack of association between children not tolerating AZA and *TPMT* and *ITPA* polymorphisms.

In accordance with earlier studies we found the same *TPMT* allele frequencies.(16,17) Only one patient in whom AZA was stopped due to adverse events was found to be a heterozygous mutant for the *TPMT*3C* allele (compound with heterozygous *ITPA IVS2)*. The other 10 patients with adverse events had wild type *TPMT* alleles. Kader et al. earlier showed that adverse events were not found to correlate with TPMT activity in 22 AZA treated paediatric IBD patients. *TPMT* alleles were not assessed in this cohort.(18)

Furthermore no relation could be demonstrated in our study between *ITPA* polymorphisms and intolerance for AZA. Only one out of 11 patients who stopped AZA due to adverse

events had a mutation in the gene encoding the *ITPA* enzyme. The Marinaki study described a significant association of the *ITPA 94C>A* missense mutation with adverse events in 62 patients, whereas our group consisted of only 11 patients with such drug reactions.(9) The fact that we don't find an association with adverse events could be explained by the fact that our study is underpowered. However, a very important finding in our study is that two of our patients were homozygous for *ITPA 94C>A* mutation, but tolerated AZA well in the standard dose (2-2.5 mg/kg). Since patients homozygous for the *94C>A* missense mutation have zero erythrocyte ITPase activity,(9) this total absence apparently does not lead to AZA toxicity. This observation is supported by the Gearry study who recently analysed 73 adult IBD patients who had experienced AZA associated adverse events and 74 control patients (tolerating AZA therapy for a minimum of 6 months) for the *ITPA 94C>A* polymorphisms.(19) No significant difference, nor even a trend to a difference, in the ITPA allele frequency between patients experiencing adverse events to AZA and controls was found.

Based on these data there is no necessity for and utility of assessment of *ITPA* polymorphisms prior to starting AZA. However, since this study was performed in paediatric patients with consequently a low number of patients, more studies are needed to confirm these results. Assessing TPMT enzyme activity or *TPMT* genotype prior to starting AZA could avoid leucopoenia in a homozygous *TPMT* patient. Three hundred patients would need to undergo TPMT assessment to detect one patient at short term risk.(20) Indeed one can argue that the potential severity of myelosuppression in one in 300 patients justifies screening. However, since leucopoenia in the majority is not caused by mutant *TPMT* homozygosity, there is a necessity for repeated blood cell counts anyway. In case repeated blood cell counts are performed TPMT assessment is not necessary.

In conclusion, AZA is an important maintenance drug in paediatric IBD. In children who do not tolerate this drug, we were not able to demonstrate an association with polymorphisms in the *ITPA* or *TPMT* gene. Other causes for AZA intolerance remain to be elucidated.

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Chapter 5

Azathioprine maintains first remission in newly diagnosed paediatric Crohn's disease

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Summary

6-Mercaptopurine (6-MP) maintains remission in paediatric Crohn's disease (CD). Azathioprine, a prodrug of 6-MP, is used for maintenance of remission of CD in Europe. We evaluated to what extent azathioprine is used in newly diagnosed paediatric CD patients and whether maintenance of remission differed between patients using azathioprine or not. Charts of children (diagnosed: 1998–2003, follow-up ≥ 18 months) were reviewed. Active disease was defined as Paediatric Crohn's Disease Activity Index (PCDAI) > 10, or systemic corticosteroid use. Remission was defined as PCDAI ≤ 10, without use of corticosteroids. Eighty-eight children (55 boys/33girls, age 12±3 yr.) were included. Seventy-two patients (82%) received azathioprine during the follow-up period (38±17 months). Patients diagnosed after 2000 received azathioprine significantly earlier during the course of disease, compared to those diagnosed earlier (median, at 233 vs. 686 days; $p<0.05$). At initial presentation, moderate-severe disease activity and prescription of corticosteroids were more prevalent in patients using azathioprine compared with non-azathioprine patients (75% vs. 52%; $p<0.05$; and 89% vs. 58%; $p<0.005$, resp.). Duration of corticosteroid use was longer in patients receiving azathioprine (232 vs. 168 days; $p<0.005$). Median maintenance of first remission in patients who initially used corticosteroids, however, was longer in patients receiving azathioprine, compared with non-azathioprine patients (PCDAI exceeding 10: 544 vs. 254 days, $p=0.08$; corticosteroid free: 575 vs. 259 days, $p<0.05$; resp.). We conclude that, since 2000, azathioprine is being introduced earlier in the treatment of newly diagnosed paediatric CD patients. The use of azathioprine is associated with prolonged maintenance of the first remission.

Introduction

The role of azathioprine (AZA) or of its active metabolite 6-mercaptopurine (6-MP) in the treatment of paediatric inflammatory bowel disease (IBD) has become a matter of debate, in particular with respect to timing of introduction and effect on modifying the course of disease.(1) The use of AZA or 6-MP treatment, for example in newly diagnosed paediatric patients with Crohn's disease (CD) has become more widely accepted by paediatric gastroenterologists in the USA.(2,3) In general, there seems to be a trend in treatment of CD towards a "top down" approach with early introduction of suggested "disease-modifying drugs", like AZA or 6-MP.(4) Markowitz et al. showed in a multicentre randomized controlled trial that 6-MP spared corticosteroid use and maintained remission in children with newly diagnosed CD.(5) The efficacy of AZA or 6-MP for paediatric CD has also been assessed in small or retrospective studies.(6-11) There is, however, no consensus with respect to the timing of introduction of AZA or 6-MP.(12) A recent international survey among 167 paediatric gastroenterologists showed significant regional differences in treatment given to children diagnosed with CD.(3)

In The Netherlands, the incidence of paediatric IBD is 5.2 per 100.000.(13) It is unclear to what extent newly diagnosed paediatric CD patients are treated with AZA and whether there has been a trend towards its earlier introduction in recent years. We aimed to determine the timing of introduction of AZA in newly diagnosed paediatric CD patients over the years 1998-2003, and whether the use of AZA was associated with the maintenance of first remission.

Materials and methods

Study patients

This retrospective study was conducted on the charts of paediatric CD patients treated in 3 academic paediatric hospitals in Groningen, Rotterdam and Amsterdam, The Netherlands. Data were obtained from paediatric CD patients, who were diagnosed between January 1st, 1998 and July 1st, 2003, and from whom a follow-up of at least 18 months up was available. The diagnosis CD had to be clearly established based on endoscopic, histopathological and/or radiological examinations. Data were collected ultimately until January 1st, 2005, or earlier in case patients reached the age of 18, or were transferred to the adult gastroenterology department.

Study design

Patients were selected using the hospital patient databases. A single investigator collected data from both electronic and paper charts. Age, sex, weight, height, localization and severity of disease at first presentation were recorded. Localization of disease was based

on endoscopic, histopathological and radiological criteria. Severity of disease activity was assessed by calculating the Paediatric Crohn's Disease Activity Index (PCDAI) from the charts. The PCDAI is a validated index, which includes symptoms, physical examination and laboratory items. A PCDAI ≤ 10 was defined as no disease activity, a PCDAI from 11-30 as mild activity and a PCDAI > 30 as moderate-severe disease activity.(14) When in doubt, because of missing data, the abbreviated clinical index developed by Loonen et al. (15,16) or a modified Harvey-Bradshaw score (17) was calculated. Active disease, initial and relapse, was determined using two definitions: PCDAI > 10 or systemic corticosteroid use.(18) The start of remission was defined as a PCDAI ≤ 10 without use of corticosteroids. Duration of remission was determined using two definitions: time until corticosteroids were started and time until the PCDAI exceeded 10. Medication regimens were recorded (type, dose, time of initiation, period of prescription and side effects), as well as surgical interventions. Height and weight were expressed as height-for-age Z-score (standard deviation score) and weight-for-height Z-score, respectively. Only patients whose height and/or weight had been reported within 2 months from initial presentation were included.

All clinical parameters (disease activity, remission, steroid use) were assessed for the patients who were prescribed AZA as well as the patients who did not receive AZA. Only patients who used AZA for more than 3 months were included in the AZA patient group. For analysis of remission more than 90 days of follow-up had to be available after corticosteroid-free remission was reached. Comparison of duration of remission with respect to AZA use was done for all patients, as well as for those patients who were prescribed corticosteroids during their initial period of active disease. If AZA was prescribed to patients while in remission, they were switched to the AZA group for remission analysis.

Statistical analysis

Data were collected in Microsoft Access 2003 (Microsoft Corporation). Data on weight and height were collected and analyzed using Growth Analyser 3 (© 2004 Dutch Growth Foundation, Rotterdam, the Netherlands). All other data were analyzed using SPSS (version 12.0.1, SPSS Inc.). For all "time until" data, event-free intervals according to Kaplan-Meier were calculated. Data were noted as medians with 95% confidence intervals (CI). If medians were not calculable, because of the low number of events, means were used. Differences in event-free intervals were statistically compared using Log rank-analysis. Differences in categorical data, such as severity and localization of disease or initial treatment were assessed with Pearson Chi-square analysis. All differences were considered significant for $p<0.05$.

Results

Patient characteristics

In the study period, 89 patients were diagnosed with CD. One patient, who was diagnosed with an isolated skin manifestation of CD, was excluded from all analyses. This patient developed intestinal activity of CD at 67 months after initial skin manifestation, with only 12 months of follow-up available. The 88 patients were treated in Groningen (n=25), Rotterdam (n=24) or Amsterdam (n=39). Twenty of the 88 patients had initially been diagnosed and treated in a non-academic centre.

Patient characteristics at diagnosis are shown in table 1. At initial presentation the diagnosis CD was based on colonoscopy with biopsies (n=83), sigmoidoscopies (n=3), enteroclysis (n=1) or surgery (n=1). All patients, except the patient that was diagnosed during surgery, had undergone repeat colonoscopy with biopsies at least once during follow-up confirming a diagnosis of CD.

Therapy for the initial period of active disease

In table 2, the prescribed therapy during the initial period of active disease is shown. During the initial period of active disease and during the subsequent remission, therapy was often changed or doses were adjusted. These changes in therapy could either reflect insufficient therapeutic response, the occurrence of side effects or the switch from an exacerbation-based regimen towards a maintenance regimen. In the first month of active disease, aminosalicylates were the most frequently prescribed therapy, often in combination with corticosteroids (39/88 patients, 44%). AZA, on the contrary, was rarely prescribed in the first month. After the first month of active disease, however, AZA becomes the most frequently

Table 1. Characteristics of newly diagnosed paediatric Crohn's disease patients at diagnosis

No. of subjects	88
Male/Female[a]	55/33 (63/37)
Age (yr)[b]	12.3 ± 3.3
Duration of follow-up (yr)[b]	3.2 ± 1.4
Localization of disease[a]	
-Small bowel	8 (9)
-Small bowel and colon	42 (48)
-Colon	38 (43)
Perianal disease[a]	13 (15)
Height (z-score; n=84)[c]	-0.41 ± 1.03
Weight for height (z-score; n=83)[d]	-0.79 ± 1.38

[a]n (%); [b]means ± SD; [c]No height recorded at diagnosis (n=3); [d]No weight recorded at diagnosis (n=1); 1 patient was excluded from assessment of height and weight, because of short stature unrelated to her CD

Table 2. Initiated drug therapy in newly diagnosed paediatric Crohn's disease patients during the initial period of active disease

	Total prescribed		Prescribed in first month	
Drug therapy	n	%	n	%
Aminosalicylates	76	86	66	75
Corticosteroids	62	70	50	57
Enteral nutrition	24	27	17	19
Antibiotics	18	20	6	7
Azathioprine	33	38	2	2

prescribed therapy, amounting into 33/88 patients using AZA during their first period of active disease. Antibiotics were almost solely prescribed for perianal disease, as were corticosteroid enemas, which were prescribed to 6 patients during the first period of active disease. Elective surgery was performed in 14 patients during the first period of active disease, two of whom had surgery within the first month (1 hemicolectomy, 1 ileocecal resection).

Azathioprine use

To assess the prescription of AZA, patients were divided into 3 cohorts according to their year of diagnosis (1998/1999, 2000/2001 and 2002/2003). At the end of follow-up, AZA had been prescribed in about 80% of patients in each of the 3 cohorts (84%, 82% and 80% respectively). However, it should be realized that the mean length of follow-up differs (1998-1999: 55 months; 2000-2001: 38 months; and 2002-2003: 26 months, respectively; $p<0.001$). Figure 1 shows that AZA was prescribed earlier in the course of disease to patients diagnosed after January 1st, 2000, as compared to patients diagnosed before January 1st, 2000. Median time (95% CI) until prescription in the different groups was: 1998/1999: 686 days (229-1143), 2000/2001: 214 days (40-388) and 2002/2003: 238 days (137-339); $p<0.05$.

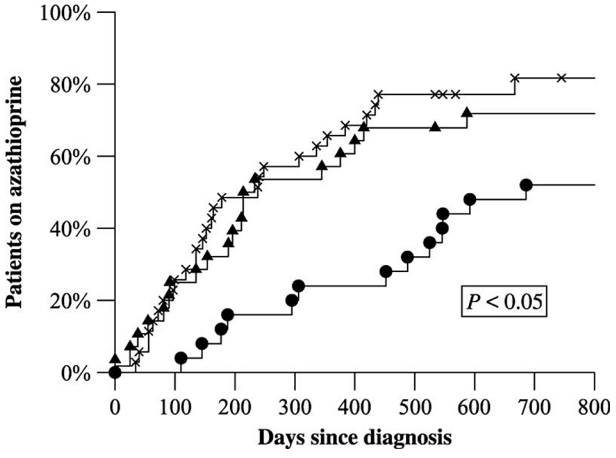

Figure 1. Prescription of azathioprine during the first 2 years after diagnosis in paediatric Crohn's disease patients, diagnosed in three cohorts: 1998/1999 (●, n=25), 2000/2001 (▲, n=28) and 2002/2003 (**X**, n=35). Data on cohort 1998/1999 are statistically different from the other two cohorts ($p < 0.05$).

At the end of follow-up 72 patients (82%) had been prescribed AZA. Median follow-up during use of AZA was 21 months (range: 2-58 months). Side effects which required changes in the therapeutic regime were observed in 12 patients (17%), requiring dose reduction in 3 patients and discontinuation of AZA in 9 patients. Reasons for dose reduction were: leucopoenia (white blood cells < 4000 mm^3; n=1), hair loss (n=1) and increase of amylase (n=1). Discontinuation of AZA was required because of gastrointestinal problems (n=4), leucopoenia (n=3), pancreatitis (n=1) and increase of amylase (n=1).

Initial period of active disease and first remission

During the initial period of active disease 33 out of the 88 patients were prescribed AZA. Table 3 shows the characteristics of patients with AZA (AZA patients) and those without AZA (non-AZA patients). Duration of the initial period of active disease, according to the PCDAI was similar in both groups (median (95% CI); AZA patients: 70 days (41-99) vs. non-AZA patients: 62 days (53-71); p=0.19). However, duration according to corticosteroid use was longer in AZA patients (median (95%CI); AZA patients: 232 days (109-355) vs. non-AZA patients: 168 days (135-201); p<0.005). AZA was discontinued in two patients before corticosteroid free remission was reached, in either case because of side effects. Four of the AZA patients did not reach corticosteroid free remission (range of follow-up: 603-1302 days).

Table 3. Characteristics of newly diagnosed paediatric Crohn's disease patients treated with or without azathioprine during the initial period of active disease

	Azathioprine		Non-azathioprine		
Variable	n	%	n	%	p-value
No. of subjects	33		55		
Male/Female	20/13	61/39	35/20	64/36	0.78
Severity of disease activity at diagnosis					
- PCDAI ≤ 30	6	18	27	49	0.004
- PCDAI > 30	27	82	28	51	
Localization of disease at diagnosis					
-Small bowel	4	12	4	7	
-Small bowel and colon	12	36	30	55	0.25
-Colon	17	52	21	38	
Initiated drug therapy					
-Aminosalicylates	26	79	50	91	0.11
-Corticosteroids	32	97	30	55	<0.0001
-Enteral nutrition	13	39	11	20	0.05
-Antibiotics	8	24	10	18	0.50
-Infliximab	4	12	-	-	0.008
-Methotrexate	2	6	-	-	0.07

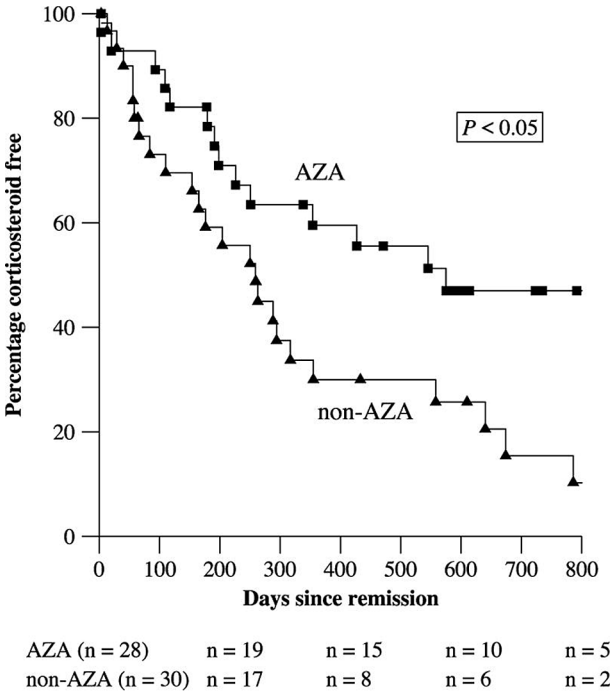

Figure 2. Maintenance of corticosteroid free remission in paediatric Crohn's disease patients with (●, n=28) or without (▲, n=30) AZA therapy, after the onset of the first remission. Data concerns children, who used corticosteroids before the first remission, i.e. during the initial period of active disease. 'n=' indicates the number of patients that were still corticosteroid free. AZA = azathioprine.

Five patients were prescribed AZA during their corticosteroid free remission. One patient (no AZA) was excluded from remission analysis, for only 63 days of follow-up remained after corticosteroid free remission was reached. In the first period of active disease a larger fraction of AZA patients had moderate-severe disease, used corticosteroids and used infliximab. Analysis showed that, according to the PCDAI, AZA patients had longer remissions than non-AZA patients (medians; AZA patients: 575 days vs. non-AZA patients: 288 days; $p<0.05$). When duration of remission was assessed according to corticosteroid use AZA patients maintained remission longer, but the difference did not reach statistical significance (mean (95% CI); AZA: 913 days (686-1141) vs. non-AZA: 665 days (480-850); $p=0.07$).

Both groups who had used corticosteroids during the initial period of active disease (AZA patients, n=28; non-AZA patients, n=30) were comparable with respect to localization and severity of initial disease and initiated drug treatment. As shown in figure 2, AZA patients had longer corticosteroid free periods than non-AZA patients (medians: AZA patients 575 days vs. non-AZA patients, 259 days; $p<0.05$). Infliximab and/or methotrexate were used by some patients concomitantly. When these patients were excluded from the analysis (AZA patients, n=3), the difference in favour of patients using AZA remained significant ($p<0.05$). In the 12 months after reaching the first remission, a profoundly larger fraction of AZA patients had maintained a corticosteroid free period, compared to non-AZA patients (15/26 vs. 8/28; $p<0.05$). When duration of remission was assessed according to the PCDAI, remission was also maintained longer in AZA patients, but the difference was not statistically

significant (medians (95% CI); AZA patients: 544 days (275-813) vs. non-AZA patients: 254 days (164-344); p=0.08).

Discussion

Our data indicate that during a mean follow-up of 3.2 yr, AZA is used in more than 80% of newly diagnosed paediatric CD patients, but rarely within one month after diagnosis. A profoundly beneficial effect of AZA on the maintenance of first remission was demonstrated, even though AZA was primarily prescribed in paediatric CD with a more severe initial presentation.

AZA maintained first remission, irrespective whether corticosteroids were prescribed during the initial period of active disease or whether disease activity was defined clinically (PCDAI) or based on use of corticosteroids. To avoid the possible effect of the use of corticosteroids on duration of remission, we chose to include their use in our definition of remission. Whether the effect is real, however, is still a matter of debate.(19-21) Retrospective studies are sensitive to bias, such as referral bias or unequal distribution of treatment over patients with different disease severity. Indeed, AZA appeared to be prescribed primarily in newly diagnosed CD patients with a moderate-severe disease activity score at diagnosis and who used corticosteroids more often and longer. Upon correction for this bias in treatment, however, similar beneficial results for AZA were found: patients who were prescribed corticosteroids in the initial period of active disease were evenly distributed with respect severity of disease, but nevertheless maintained first remission longer when using AZA.

In retrospective chart studies, it is not always possible to extract all data precisely from the charts. In our study the data considering the evaluation of disease activity and the use of corticosteroids required special attention. To avoid bias on this area as much as possible, we attempted to use multiple assessments of disease activity and to use a standard regimen of corticosteroid use, available to all clinicians who treat paediatric CD.

The efficacy of treatment with AZA and 6-MP in adults with CD has been well established. In children, a hallmark multicentre, randomized controlled trial by Markowitz et al. showed that, in newly diagnosed paediatric CD patients 6-MP was effective in maintaining remission, in sparing corticosteroid usage and in preventing steroid-dependency.(5) Efficacy in inducing remission was not shown. Other data on AZA use in children with CD or ulcerative colitis can only be derived from small studies or retrospective reports.(6-8,10,11) Our study shows the potency of AZA for treatment of paediatric CD in a retrospective, non-protocolised study design. Our present results with respect to maintenance of remission were not as strong as those observed by Markowitz et al., concerning 6-MP in a randomized controlled trial.(5) Conclusions from this comparison, however, cannot be drawn. Not only because prospective and retrospective studies are difficult to compare, but also because the time point of evaluation of treatment was defined differently. In the study by Markowitz et al. the results of treatment with 6-MP were evaluated 18 months after diagnosis, while in our study efficacy

of maintenance of remission was recorded at a time point at least 90 days after remission was reached.(5)

Our data provides indirect information for the present debate whether active disease in (paediatric) CD should be treated by a "step up" or "top down" therapeutic strategy.(22) It has been suggested that a therapeutically aggressive approach early in the course of the disease can improve its clinical course.(5,22) Our present data could be interpreted to favour a more aggressive approach early in the course of the disease: the duration of first remission could be prolonged by the (early) introduction of AZA, namely during the initial period of active disease. In general, the use of AZA in paediatric IBD is not only considered efficacious, but also safe.(2,6,7,23) Compared to these studies, our study showed comparable rates with respect to dose reduction and discontinuation of AZA because of side effects (4% and 13%, respectively). It should be emphasized, however, that only limited information is available on the risks and benefits of long-term use of immunosuppressants for paediatric CD. This information would allow a more elaborate benefit – risk analysis for a "step up" or "top down" strategy.

In our study AZA is only seldom prescribed within one month after diagnosis. This reflects the outcome of an international survey, which showed that only 4.2% of paediatric gastroenterologists in Western Europe see newly onset of CD as an indication for the use of AZA, contrary to their colleagues in the USA (20%).(3) It is interesting to see that the only study, that has also assessed initial treatment in newly diagnosed paediatric CD patients, conducted in Great Britain and Ireland, showed remarkably comparable rates with respect to initiated treatment within one month.(24) In monthly mailings that were sent out to register newly diagnosed paediatric IBD patients, initiated treatment was also registered. More or less similar use of aminosalicylates (69%), systemic corticosteroids (56%), AZA (6%), antibiotics (12%) and surgery (3%) were seen in paediatric CD patients. The only difference was seen in nutritional therapy (33% in Great Britain and Ireland vs. 19% in our study). This possibly indicates that, though a general lack of consensus on major therapeutic strategies is recognized, newly diagnosed paediatric CD patients, at least in Western Europe, are treated in a similar way.(12)

In conclusion, we report the results of a multicentre retrospective assessment of the use of AZA in The Netherlands and its relation to maintenance of first remission in newly diagnosed paediatric CD patients. Although AZA is only seldom prescribed within the first month after diagnosis, there has been a shift towards an earlier introduction of AZA in the course of disease. AZA is primarily prescribed for paediatric CD with a moderate-severe initial presentation and irrespective of its use in more severe disease, AZA has shown to maintain first remission in paediatric CD.

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Chapter 6

Infliximab use in children and adolescents with Inflammatory Bowel Disease

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Summary

Infliximab is a chimeric monoclonal antibody (75% human, 25% murine) against tumour necrosis factor-α, a cytokine with a central role in the pathogenesis of inflammatory bowel disease.

Large randomized controlled trials have shown efficacy and safety of infliximab for the induction and maintenance of remission in adult patients with active Crohn's disease. In children and adolescents mostly small, non-randomized and non-placebo controlled studies supported the notion that infliximab is a very potent drug in a population that is not responding to standard therapies. The safety of infliximab is of major concern, and the most frequent severe adverse events are related to severe infections and re-activation of tuberculosis. Other complications such as autoimmune disorders and aplastic anaemia occur infrequently. The association of infliximab with development of cancer or lymphomas remains unclear. Non-life threatening infusion reactions do occur rather frequently and seem to be related to formation of human antichimeric antibodies. Immunomodulation (azathioprine, methotrexate) functions synergistic to infliximab and because it decreases the formation of human antichimeric antibodies it should be instituted before infliximab therapy is started. Indications for infliximab treatment are therapy resistant luminal CD (none or insufficient efficacy of conventional treatment) and therapy resistant fistulas. An efficient remission induction strategy consists of 3 initial infliximab infusions at 0, 2 and 6 weeks in a dose of 5 mg/kg to sustain remission. Patients needing maintenance therapy are subsequently treated with an infliximab infusion, every 8 weeks. There are indications that the early stages of CD might be more susceptible to immunomodulation and the natural history of CD might be altered by introduction of infliximab early in the disease process instead of waiting until conventional therapy has failed. A major point of discussion is whether infliximab maintenance treatment should be episodic (on demand) or scheduled, and when infliximab therapy can be discontinued.

Introduction

Infliximab (Remicade®) is a chimeric monoclonal antibody (75% human, 25% murine) that avidly binds human tumour necrosis factor-α (TNF-α), a cytokine with a central role in the pathogenesis of many inflammatory processes.(1) TNF-α expression is increased in the inflamed intestinal mucosa of both adults and children with active Crohn's disease (CD).(2,3) Infliximab inhibits the bioactivity of TNF-α by directly binding to the cytokine and also modulates the function of TNF-α-producing cells. Infliximab was initially developed for treatment of sepsis syndrome, but was found to have a remarkable anti-inflammatory effect in a child with severe Crohn's colitis.(4) Following clinical studies in adult patients with CD that confirmed clinical benefit, infliximab was approved by the U.S. Food and Drug Administration (FDA) in 1998 for short-term treatment of moderately to severely active luminal and fistulizing CD. Hereafter, infliximab maintenance therapy in luminal CD was approved in 2002 and in fistulizing CD in 2003. Maintenance treatment of luminal CD was approved by European Medicines Agency (EMEA) in 2003. In 2005 infliximab also received approval from the FDA for treatment of patients with UC, in 2006 approval is expected to be granted by EMEA (data obtained from Centocor). It is ironical that, although 16 years ago the first patient ever treated with infliximab was a 13-year-old child, infliximab only very recently has been approved for treatment of moderately to severely active paediatric CD.(5)

Mechanism of action

TNF-α is produced as a 26-kilodalton transmembrane precursor protein, which requires specific proteolytic cleavage to release the biologically active 17-kilodalton form. The protease responsible for the cleavage is TNF-α converting enzyme (TACE), a membrane anchored multidomain metalloproteinase.(6) Cleavage probably takes place after aggregation of membrane-bound form of TNF-α as trimolecular complexes. Secreted TNF-α can bind to two cell surface TNF receptors (TNF-R1 and TNF-R2) and membrane-bound TNF can also induce responses in target cells, by engaging TNF-R2. Apart from inducing activation of the target cell through TNF-R2, these cell to cell interactions also cause activation of the cell that expresses membrane bound TNF, through "reverse signalling".(7,8) Infliximab is capable of neutralizing soluble TNF-α, and there is little doubt that this has anti-inflammatory activity. In addition, part of the efficacy of infliximab in Crohn's disease may be based on its binding to membrane-bound TNF-α causing reverse signalling. Cells that express membrane-bound TNF-α include activated lymphocytes from peripheral blood and the lamina propria of CD patients and one of the effects of infliximab binding is induction of apoptosis of activated T- cells (7,9,10). Three polymorphisms in apoptosis genes potentially influence response to infliximab in luminal and fistulizing CD patients.(11) Response to infliximab may be determined by other physiologic or genetic reasons. One of the effects of infliximab therapy is activation (through phosphorylation) of p38α, a mitogen-activated protein kinase (MAPK) that regulates the expression of pro-inflammatory cytokines. A direct effector target of p38α is activating transcription factor-2 (ATF-2) and the secondary target heat shock protein-27

(Hsp-27), and only responders to infliximab demonstrated phosphorylation of ATF-2 and Hsp-27. TNF-α and NOD2 gene polymorphisms do not predict response to anti-TNF treatment, but variations in several genes in or outside the TNF signalling cascade show a relationship with response.(8,12-14) Unfortunately, none of these genetic associations is strong enough to predict efficacy in the individual patient.

Clinical efficacy in adults with active CD

Several randomized controlled trials have assessed the clinical efficacy of infliximab in adult patients with active Crohn's disease (table 1). In 1995 a multicentre randomized placebo-controlled double-blind clinical trial in 108 patients with moderate to severe CD resistant to treatment was reported, the primary endpoint of this study being clinical response, defined as a reduction of 70 or more points in the CDAI [Crohn's Disease Activity Index, a validated index of CD activity in adults (15)] at four weeks after infliximab or placebo, without change in concomitant medication. A positive clinical response was found in 65% ($p<0.001$ compared to placebo) and clinical remission (CDAI<150) in 33% of patients ($p<0.005$ compared to placebo) after one infliximab infusion.(16) In 1996 a multicentre randomized placebo-controlled double-blind trial was performed in 94 CD patients with fistulas. Patients were given 3 infliximab infusions (at 0, 2 and 6 weeks) or placebo. The primary endpoint was a reduction of 50% or more from base-line in the number of draining fistulas observed at two or more consecutive visits. Reduction of 50% or more was seen in 62% of patients against 26% of patients who received placebo.(17) In 1999 another large, international placebo-controlled, randomized double-blind trial, ACCENT I, was completed that included 573 patients with moderate to severe CD, that were treated with a single infliximab infusion. Patients were randomized to repeated placebo infusions at 2, 6 weeks and subsequently every 8 weeks (group I); or to repeated infliximab infusions 5 mg/kg at 2, 6 weeks and subsequently every 8 weeks (group II) or to repeated infliximab infusions 10 mg/kg at 2, 6 weeks and subsequently every 8 weeks (group III). The primary endpoints were the proportion of patients who responded at week 2 and were in remission (CDAI<150) at week 30 and the time to loss of response up to week 54 in patients who responded. At week 30, 21% of patients treated with placebo, 39% of patients treated with infliximab 5 mg/kg and 45% of patients treated with infliximab

Table 1. Large, randomized, placebo-controlled, double-blind trials on efficacy of infliximab in adults with CD

Study	Number	Population	Infliximab
Targan et al.(16)	108	luminal disease	1 infusion
Present et al.(17)	94	fistulizing disease	0, 2, 6 weeks
Hanauer et al.(18)	573	luminal disease	0, 2, 6 weeks, every 8 weeks
Sands et al.(19)	306	fistulizing disease	0, 2, 6 weeks, every 8 weeks

10 mg/kg were in remission. The median time to loss of response was 46 weeks in groups II and II combined compared with 19 weeks in group I (p=0.0002). The authors concluded that CD patients responding to an initial dose of infliximab are more likely to be in remission at weeks 30 and 54, to discontinue corticosteroids, and to maintain response for a longer time if infliximab treatment is maintained every 8 weeks.(18) ACCENT II, a randomized placebo-controlled trial evaluated infliximab maintenance therapy in 306 adult CD patients with one or more draining abdominal or perianal fistulas of at least three months duration. Patients received induction therapy (5 mg/kg at weeks 0, 2 and 6) and were then randomized to infliximab maintenance therapy or placebo. The primary endpoint was the time to loss of response among patients who had response at week 14. Loss of response was defined as recrudescence of draining fistulas, need for other or additional medication for persistent or worsening luminal disease activity, need for a surgical procedure or discontinuation of study drug because of lacking efficacy. Patients receiving infliximab maintenance therapy had significantly longer median time to loss of response compared to placebo (> 40 weeks vs.14 weeks, p<0.001).(19)

Clinical efficacy in children and adolescents with active CD

In children and adolescents mostly small, non-randomized and non-placebo controlled studies have been performed (table 2). Eleven non-controlled, both prospective and retrospective studies have been reported in which 15-21 children with therapy resistant CD were treated with 1-6 infliximab infusions.(20-30) Almost all patients were treated with infliximab at a dose of 5 mg/kg. Baldassano et al. performed a dose-finding study in 21 CD patients between the ages of 11 and 17 years.(30) Pharmacokinetic profile in paediatric patients was found to be similar to that in adults. This consistency indicates that the 5 mg/kg dose recommended for adults maybe the appropriate dose in children as well. The majority of these patients were also treated with azathioprine or methotrexate. Clinical improvement and tapering of steroid use was reported in the majority of patients, with quite homogeneous findings. Relapses of disease activity following discontinuation of infliximab treatment were common.(20,21,26, 28,29) Interestingly, in an open-label, prospective clinical trial Kugathasan et al. found that the therapeutic effect of infliximab was sustained longer in paediatric patients with early CD

Outcome	p-value
65% clinical response, 4 weeks after 1 infusion	0.001
55% minimally temporarily closure	0.001
21% (placebo); 39% (5 mg/kg);	0.003 (5 mg/kg vs placebo)
45% (10 mg/kg) in remission at week 30	
11% (placebo); 25% (5 mg/kg);	0.007 (5 mg/kg vs placebo)
33% (10 mg/kg) in remission at week 14 to 54	
19% complete absence of draining fistulas	<0.001

Table 2. non-randomized, non-placebo controlled studies on efficacy of infliximab in children with CD

Study	Number	Population	Infliximab
Kugathasan et al.(20)	15	luminal disease	1 infusion
Lionetti et al.(22)	22	luminal and fistulizing disease	mean of 3.3 infusions
Hyams et al.(21)	19	luminal disease	1-3 infusions
Stephens et al.(23)	82	luminal and fistulizing disease	mean of 5.3 infusions
De Ridder et al.(24)	30	luminal and fistulizing disease	mean of 7.1 infusions
Wewer et al.(25)	24	luminal and fistulizing disease	mean of 4.8 infusions
Serrano et al.(26)	15	luminal and fistulizing disease	mean of 1.7 infusions
Lamireau et al.(27)	88	luminal and fistulizing disease	median of 4 infusions
Borelli et al.(28)	18	luminal and fistulizing disease	mean of 3.7 infusions
Cezard et al.(29)	21	luminal and fistulizing disease	3 infusions
Baldassano et al.(30)	21	luminal and fistulizing disease	1 infusion
one randomized open label study			
REACH (31)	112	luminal and fistulizing disease	0, 2, 6 weeks, every 8 weeks 0, 2, 6 weeks, every 12 weeks

compared to patients with long-standing CD.(20) In their retrospective study Lionetti et al. found that children diagnosed with CD for less than 1 year had a higher chance on a longer duration of response than children diagnosed with CD for more than 1 year.(22)
Stephens et al. retrospectively studied 82 children (mean age 15.3 years) with CD who were treated with an average of 5.3 infliximab infusions. As a measure of efficacy, corticosteroid use was evaluated. A significant decrease in steroid use was found, but it should be noted that only 33 out of 82 patients were receiving corticosteroids when infliximab was initiated and had adequate follow-up data on steroid use. Moreover, steroid use is a less sensitive measure of response than disease activity indices, which decreases the clinical relevance of these findings substantially.(23) Wewer et al. demonstrated that out of a total of 24 paedi-

Follow-up duration (mean, weeks)	Outcome	Safety (n=number of patients)
10	93% significant decrease of PCDAI and steroid use	intestinal strictures (n=1)
18	significant decrease of PCDAI at 16-18 weeks patients with fistulizing disease: 54% complete response, 23% partial response	infusion reactions (n=2)
12	significant decrease of PCDAI and steroid use	infusion reactions (n=3)
46.4	significant decrease of PCDAI	infusion reactions (n=12); herpes zoster infection (n=3); Listeria monocytogenes meningitis (n=1)
109	53% effective maintenance therapy	infusion reactions (n=6); death due to sepsis (n=1); general malaise (n=6); arthritis (n=1)
13 (after last infusion)	29% prolonged response, 42% infliximab dependency	infusion reactions (n=3); delayed hypersensitive reaction (n=1)
NA	69% relapse, 14.2 weeks (mean) after last infusion	rash (n=1)
13	34% in remission, 53% improvement, 13% relapse	infusion reactions (n=13); delayed hypersensitive reaction (n=2); with abscesses (n=3); intestinal strictures (n=2)
26	significant decrease of PCDAI in retreatment group	infusion reactions (n=4)
52	90% complete remission at 6 weeks; 90% relapse at 1 year	infusion reactions (n=1); sepsis (n=1); autoantibody formation (n=8); increased EBV PCR (n=1)
12	100% clinical response, 48% clinical remission	serious infection (n=2)
54	56% clinical remission 24% clinical remission	infusion reactions (n=7); autoantibody formation (n=3); serious infections (n=7)

atric CD patients (median age 15.4 years) 29% was unresponsive to infliximab, 29% had a prolonged response after discontinuation of infliximab therapy, while 42% was dependent on infliximab reinfusion.(25) One study with a mean follow-up of 25.3 months demonstrated that approximately half of the patients (mean age 14.1 years) with refractory paediatric CD became unresponsive to infliximab maintenance therapy.(24)

Recently, a randomized, multicentre, open-label study (REACH) was conducted with the aim to evaluate the safety and efficacy of infliximab in paediatric patients with moderate to severe CD. Patients (n=112) aged 6 to 17 years with moderately to severely active CD, despite treatment with an immunomodulator with or without corticosteroids, received infliximab 5 mg/kg at weeks 0, 2 and 6. One hundred three patients were randomized at week 10 to receive inf-

liximab every 8 weeks (n=52) or every 12 weeks (n=51) through week 46. Eighty-eight % of patients treated with infliximab 5 mg/kg at 0, 2 and 6 weeks achieved the primary end point of the trial, clinical response at week 10, which was defined as a decrease from baseline of at least 15 points in the Paediatric Crohn's Disease Activity Index (PCDAI), and a PCDAI less than or equal to 30. At 54 weeks, 56% of patients (29 of 52) receiving infliximab maintenance every 8 weeks were in clinical remission as assessed by a PCDAI score of less than or equal to 10, compared with 24% (12 of 51) in the every 12-week maintenance group ($p<0.001$). Maintenance therapy every 8 weeks was superior to every 12 weeks.(31) These data suggest that the effects of infliximab treatment of children may be better than the results in adult patients, both for remission induction and for maintenance therapy.

Growth retardation and infliximab

Growth retardation is a common complication of Crohn's disease in children and adolescents and restoration of normal growth is a well-accepted marker of therapeutic success. As yet, only few studies have examined the effects of infliximab therapy on linear growth, but restoration of normal linear growth velocity and increased height centile for patients treated prior to or in early puberty was frequently reported.(28,29,32) In contrast, restoration of growth was not found in a Danish cohort.(25) It should be noted that these studies have not included sufficient patients to allow any definitive conclusions about growth improvement and infliximab treatment of CD.

Clinical efficacy in adults with active ulcerative colitis

From 2002 to 2005 two large international randomized placebo-controlled trials, ACT 1 and ACT 2 were conducted in adult outpatients with UC (364 patients in each study), refractory to corticosteroid therapy (no improvement of symptoms of UC after receiving the equivalent of at least 40 mg of prednisone daily, administered orally for at least 2 weeks or intravenously for at least one week).(33) Clinical response was defined as a decrease from base-line in the total Mayo score of at least 3 points and at least 30%, with an accompanying decrease in the sub score for rectal bleeding of at least 1 point or an absolute subscore for rectal bleeding of 0 or 1. In ACT 1 the clinical response rate at week 54 was 46% for patients treated with infliximab (5 mg/kg), which was significantly higher than for the placebo-treated patients ($p<0.001$). In ACT 2 clinical response rate at week 30 was 47% which also was significantly higher for the patients treated with infliximab 5 mg/kg versus placebo ($p<0.001$). Furthermore, mucosal healing was observed significantly more frequent in the infliximab treated patients. These two studies have firmly established the efficacy of infliximab in treating active UC in outpatients. Recently, another randomized double-blind placebo-controlled study evaluating the efficacy of infliximab was performed in UC patients with an acute severe or moderately to severe attack of UC that did not respond to high doses of corticosteroids intravenously. Significantly more patients were kept from colectomy by infliximab rescue therapy than placebo during a follow-up of 6 months (17 of 24 patients in the infliximab group and 7 of 21 patients in the placebo group).(34) Based on ACT I and ACT

II, the FDA has approved infliximab for treatment of mild to moderate UC, but the label does not include rescue therapy of fulminant UC.

Clinical efficacy in children and adolescents with active UC

Only small, retrospective cohort studies have been performed in severe to moderately severe paediatric UC patients. Mamula et al. reviewed 17 paediatric UC patients who received infliximab. Disease activity in these patients varied from mild to fulminant. Clinical improvement defined as avoidance of colectomy or a lack of need for rescue medication was seen in approximately 75% of patients. Fourteen of 17 (82%) patients responded to the initial dose of infliximab (5 mg/kg), and 10 of 16 (63%) patients had sustained response on infliximab maintenance therapy. Two patients lost response within 9 months of the first infliximab dose and required colectomy.(35,36) Russell et al. reviewed 14 paediatric UC patients treated with infliximab. Nine patients showed clinical response defined as a drop of minimally 2 points on the Lichtiger Colitis Activity Index.(37) Eidelwein et al. retrospectively studied 12 paediatric UC patients treated with infliximab. Response was defined as complete resolution of symptoms based on physician assessment. All patients were considered short-term responders and 8 patients long-term responders (median follow-up 10.4 months).(38) In each of these 3 studies patients with fulminant colitis, acute exacerbation of colitis, steroid-dependent or steroid-refractory colitis were included, rendering the patient populations very heterogeneous.

In view of the lack of relevant studies, it is unclear whether infliximab treatment prevents colectomy in paediatric ulcerative colitis in the long-term, and such patients should only be treated with infliximab within trials.

Extraintestinal manifestations

Many case reports and some open label studies have been published on the use of infliximab for treating adult patients with extraintestinal manifestations. Herfarth et al. performed an open label prospective, multicentre clinical trial in 153 adult patients with CD. Patients with arthritis or arthralgia were graded by the treating physician on a four-point scale as severe, moderate, mild or none. Thirty-six out of 59 patients (61%) with signs of arthritis or arthralgia improved by at least one point in the symptom score ($p<0.001$) at week 12 after infliximab infusion.(39) Kaufman et al. performed an open label study to prospectively assess the effect of infliximab on extraintestinal manifestations in adults with CD. Twenty-two CD patients and one UC patient presented with arthralgia, synovitis, inflammatory back pain, pyoderma gangrenosum and/or aphthous stomatitis. About 70% of the patients significantly improved after one infliximab infusion.(40) Acute and chronic uveitis are associated with IBD and both types respond to infliximab therapy.(41)

Approximately 7-24% of paediatric IBD patients experience extraintestinal manifestations (involving joints, skin, eyes, etc.).(42) Treatment of these manifestations includes topical and systemic corticosteroids and immunomodulatory agents, response however is often poor. Anecdotal reports suggest that skin manifestations in children with CD may respond to inflix-

imab. Escher et al. described a 10-year-old boy with cutaneous, metastatic CD of the penile and scrotal skin. Initial treatment of oral prednisolone combined with azathioprine or cyclosporine only transiently reduced the genital lymphedema. Treatment with infliximab infusions 5 mg/kg at week 0, 4 and 12 decreased swelling and redness.(43) Batres et al. described a 13-year-old girl with longstanding CD complicated by peristomal pyoderma gangrenosum. Despite repeated surgery because of prolapse of the ileostomy and medical treatment with mesalamine and metronidazole the ostomy site healed incompletely and the abdominal wall ulcerated significantly. The patient received infliximab infusion of 5 mg/kg at week 0, 2 and 10 and the abdominal wall wound healed almost completely within 2 weeks of the first infusion. Four months after the first infusion the peristomal pyoderma had not recurred.(44) Kugathasan et al. described 4 children (7- to 16-year old) with CD with dermatologic manifestations: pyoderma gangrenosum, orofacial involvement, erythema nodosum and idiopathic lymphedema. These manifestations were unresponsive to conventional therapy but had rapid and sustained response to 2 or 3 infliximab infusions.(45) Hence, in children with severe extraintestinal complications that do not respond to standard therapy, a therapeutic trial of infliximab should be considered.

Infliximab safety

Although approximately 270,000 patients with CD are treated with infliximab, there are few well designed long-term studies on its safety. Colombel et al. investigated the safety profile in 500 adult patients with CD treated with 2211 infusions in total. In 6.0% of patients severe adverse events possibly related to infliximab infusions were seen. These adverse events contained severe infections in 15 patients. Ten patients died, 5 out of these 10 (0.8%) were judged as likely or possibly related to infliximab. One of these patients had a contra-indication to infliximab (a persistent abdominal abscess), 6 of the 10 had severe co morbidity such as liver cirrhosis and coronary heart disease.(46) The interpretation of the relationship with infliximab treatment is difficult, because most of these patients were severely ill and were also treated with corticosteroids and immunomodulatory drugs. The TREAT registry is a prospective, observational, multicentre, long-term registry including 6290 CD patients with nearly 15,000 patient-years of follow-up evaluating pre-specified safety-related outcomes. Half of the patients received infliximab, whereas half received other therapies, such as corticosteroids and immunomodulators. The incidence of malignancies in the two groups was similar (0.58 per 100 per year in infliximab patients vs. 0.53 in non-infliximab patients; RR=1.1, 95% CI=0.71-1.63), as was the incidence of lymphoma (0.06 per 100 per year in infliximab patients compared to 0.05 in non-infliximab patients; RR=1.3, 95% CI=0.36-5.03).(47,48) These data are in contrast with the observation of the Arthritis Advisory Committee. They found CD patients treated with infliximab had an 8-fold higher rate of lymphoma as compared with healthy controls. It remains unclear whether this is a result of the treatment or the underlying disease or the combination therapy with azathioprine.(49) Bongartz et al. recently assessed the extent to which anti-TNF antibody therapies in patients with rheumatoid arthritis may increase the risk of serious infections and malignancies in patients with rheumatoid arthritis

in a meta-analysis. Nine randomized, placebo-controlled trials of infliximab or adalimumab (fully humanized monoclonal TNF-α antibody) containing 3493 patients who received anti-TNF antibody treatment and 1512 patients who received placebo were included. A pooled ratio for serious infection was 2.0 (95% CI=1.3-3.1), for malignancy it was 3.3 (95% CI=1.2-9.1).(50) These data cannot be directly extrapolated to patients with inflammatory bowel diseases, in view of the much higher background malignancy risk in rheumatoid arthritis. In adult CD patients, infliximab therapy has been associated with adverse outcomes in patients with congestive heart failure.(51) Also exclusively reported in adults is the association of infliximab with rare cases of optic neuritis, seizures and demyelinating disorders, including multiple sclerosis.(52) Another concern is the development of antinuclear antibodies in patients treated with infliximab. Infliximab is an IgG1 antibody that binds TNF-α on the cell surface and induces cell lysis, which may initiate antinuclear antibody formation.(53) Vermeire et al. investigated the development of auto-antibodies 1, 2 and 3 months after infliximab infusions in adult CD patients. The cumulative antinuclear antibody incidence at 24 months was 56.8% (71 out of 125 patients), two patients developed drug-induced lupus without major organ damage and 1 patient developed autoimmune haemolytic anaemia.(53)

In several relatively small paediatric studies serious adverse events were described in 4.7% (27 out of 578) of patients, being death due to sepsis (n=1), listeria monocytogenes meningitis (n=1), serious or unusual infections such as abscess, reactivation of Epstein Barr virus and shingles (n=22) and intestinal strictures (n=3).(20-29,31,54)

Recent post-marketing surveillance data have prompted the manufacturer of infliximab Centocor inc. to report the occurrence of 8 cases of hepatosplenic T-cell lymphoma in young CD patients out of approximately 270,000 CD patients treated with infliximab (unpublished data). Hepatosplenic T-cell lymphoma is a very rare form of non-Hodgkin's lymphoma occurring mostly in adolescent and young adult males.(55) Six out of these 8 patients unfortunately had a fatal outcome. The patients ranged in age from 12-31 years and were all on concomitant therapy with azathioprine or 6-mercaptopurine. Five out of 6 patients were males. One out of these 6 patients is described in a case-report.(56) Whether development of the lymphoma is related to 6-mercaptopurine and/or infliximab remains unclear. Although the absolute risk is small this recent finding is of high concern. To our knowledge, autoimmune or neurological disorders associated with infliximab therapy have never been reported in paediatric patients. As yet, the benefits of infliximab for treatment of therapy-refractory patients with Crohn's disease seem to outweigh the risks. Severe adverse events are reported in low frequency, but may be severe and even fatal. These include severe opportunistic infections, re-activation of tuberculosis and the occurrence of malignancies, especially hepatosplenic T-cell lymphoma.

Immunogenicity

As a chimeric antibody infliximab is associated with the formation of antibodies to infliximab (ATI), formerly called human antichimeric antibodies. The presence of ATI is associated with

an increased rate of infusion reactions, delayed hypersensitive reactions and with a shortened duration of response.

Out of the 500 CD patients described by Colombel et al. 2 patients developed severe infusion reactions and 5 patients developed delayed hypersensitive reactions. Acute infusion reactions were seen in 19 patients, leading to infusion withdrawal in 9 patients. In 2 out of 9 patients the allergic reaction was life threatening and epinephrine was administered.(46)

The cumulative incidence of infusion reactions in paediatric studies were reported in 11.3% of patients (receiving 1-30 infliximab infusions), usually not leading to withdrawal of infliximab.(20-31) The percentage of infusion reactions in these studies is relatively high, but in most of these studies infliximab was often not routinely combined with immunomodulation and infusions were not introduced at an interval of 0, 2 and 6 weeks yet.

Three cohort studies determined the rate of infusion reactions in 57 children receiving 361 infliximab infusions, 111 children receiving 594 infliximab infusions and 243 children receiving 1652 infliximab infusions respectively.(54,57,58) Infusion reactions occurred in 8.5%, 8.1% and 16.5% respectively of patients and in 9.7%, 1.5% and 3.6% respectively of all infusions. Delayed hypersensitive reaction was reported in 0.7% of paediatric patients. Immunomodulation (azathioprine, methotrexate) functions synergistic to infliximab and seems to decrease the risk for antibody formation and the risk for infusion reactions.(18,59-61) Amongst others, this is illustrated in a paediatric cohort (n=34), showing ATI formation in 35.5% of patients. There was a trend toward a lower incidence of ATI formation in patients younger than 14 years of age.(61) Female gender, immunosuppressive use for less than 4 months and prior infusion reactions were identified as possible risk factors for subsequent infusion reactions. Premedication did not seem to prevent the development of infusion reactions. However, once an infusion reaction has occurred, premedication can prevent subsequent infusion reactions.(58)

Early use of infliximab and avoidance of corticosteroids

Conventional medical therapy for remission induction in IBD includes the use of corticosteroids for moderate to severe disease activity. Corticosteroid dependence is seen in 36% and steroid resistance in 20% of CD patients.(62) Long term use of steroids causes many side effects such as striae, cataract, osteoporosis, growth failure and infection.(47,63,64) Corticosteroids are not efficacious in maintaining remission, thereby increasing the risk for surgery.(65) Maintenance therapies such as azathioprine, 6-mercaptopurine or methotrexate are often indicated in patients with moderate to severe CD to maintain remission. Markowitz et al. demonstrated that the addition of 6-mercaptopurine to remission induction with corticosteroids in children with moderate-to-severe CD is steroid sparing and improves maintenance of remission.(66)

For several reasons, it may be more effective to use infliximab early in the disease process instead of waiting until conventional therapy has failed. The early stages of immune-mediated disease may be more susceptible to immunomodulation and the natural history of CD may be altered.(67,68) As mentioned earlier, in children prolonged duration of response after

infliximab therapy is reported when the drug was initiated early in the disease course.(20) A recent randomized controlled trial in 130 adults with CD compared top-down treatment with 3 infusions of infliximab and azathioprine to step-up treatment with corticosteroids. A statistically higher efficacy of infliximab if started according to the top-down strategy (remission attained in 74.5% [top-down] vs. 48.1% [step-up], p=0.006) was shown.(69) In 2001, an adolescent with CD treated with infliximab as first line therapy (without corticosteroids) achieving clinical remission was described.(70) A report of a corticosteroid naïve 12-year-old girl with severe Crohn's colitis (PCDAI 60) who was treated with infliximab and azathioprine as first line treatment (top-down versus step-up), is reported in this thesis. Eight weeks after starting treatment, she was in clinical remission and had completely healed mucosa (PCDAI 5) and 7 months later, she was still in clinical remission.(71) Recently, a small study of 10 paediatric CD patients receiving infliximab as initial therapy (in combination with azathioprine or methotrexate) versus 19 paediatric CD patients who were treated with solitary nutritional therapy and/or corticosteroids (in combination with azathioprine or methotrexate as maintenance therapy) was reported as an abstract. During the following 12 months, 1 patient treated with infliximab as initial therapy and 17 patients treated conventionally relapsed. None of the patients treated with infliximab as initial therapy underwent surgery against 4 patients treated conventionally. Infliximab as first line therapy seemed to promote long term remission and seemed to change the course of disease.(72) However, azathioprine dosage was 1.5 mg/kg and therefore underdosed in the study population, which may explain the high relapse rate in the conventionally treated group.(Cucchiara S, Oral presentation on DDW, Los Angeles 2006; ID#56)

Guidelines for infliximab treatment

Indications for infliximab treatment are therapy-resistant luminal CD (none or insufficient effectiveness of conventional treatment) and therapy resistant fistulas. Conventional therapy is not defined in the FDA approval and may include mesalazine, corticosteroids, antibiotics, solitary enteral feeding or immunomodulation (azathioprine, 6-mercaptopurine or methotrexate). Most experts consider immunomodulation given at adequate dose for a minimum period of 3 months essential before conventional treatment can be defined as failing. Before starting infliximab therapy, surgery should be considered as an alternative option, especially in ileo-coecal localization. Tuberculosis must be ruled out by chest x-ray and skin test of purified protein derivative (PPD) tuberculin. Non smoking, absence of stricturing disease and concomitant immunomodulation predict better response on infliximab.(41)

The recommended initial dose of infliximab is 5 mg/kg in an induction regimen at weeks 0, 2 and 6 weeks. Infliximab therapy can be continued on maintenance or an episodic base. Patients treated with scheduled maintenance therapy at 8-week intervals have lower ATI rates, fewer hospital stays and fewer operations. Patients responding to infliximab therapy and needing maintenance therapy are subsequently treated with an infliximab infusion every 8 weeks. Currently, immunomodulation is usually continued. However, since patients already failed on immunomodulation and taking into account the possible increased risk of lym-

phomas it might in future turn out to be a better strategy to administer infliximab as sole medication. In case immunomodulation is not used for an interval of 3 months or more corticosteroid intravenously combined with an antihistamine should be administered prior to the infliximab infusion to prevent the formation of ATI.

In case repeated relapses occur preceding the next infliximab infusion the interval of infusions can be shortened. In case of decrease of response after an infliximab infusion the dose can be doubled, since antibody formation against infliximab probably has occurred.(61) Treatment should be administered in a centre familiar with the drug, with emergency equipment for severe infusion reactions available and all patients should be monitored for up to 1 hour after the infliximab infusion. In case of non response after 3 infliximab infusions, further infliximab infusions are very unlikely to provide clinical benefit and infliximab maintenance therapy is not recommended.

Costs

Infliximab is a very costly therapy, 1 ampoule containing 100 mg costs 639.35 €, and infliximab has to be administered intravenously, in a hospital setting. As far as we know, no studies on cost efficacy of infliximab therapy in paediatric IBD have been conducted. However, in adult patients hospital admissions, surgical interventions, endoscopies and presentations at the emergency room are halved during infliximab treatment, and therefore infliximab therapy may be cost effective.(7,73)

Other anti-TNF drugs

Adalimumab (Humira) is a fully human anti-TNF-α monoclomal IgG1 antibody. Sandborn et al. performed an open label study of adalimumab in 24 CD patients who lost response or were intolerant to infliximab. Primary endpoints were ability to tolerate adalimumab and clinical response (defined as CDAI $\leq$ 100 points). All patients were able to tolerate adalimumab. Clinical response at week 12 occurred in 59% of patients. Seventy-nine% of patients required dose-escalation at week 4-6.(74) Hanauer et al. performed the CLASSIC-I trial, a randomized, double-blind, placebo-controlled, dose-ranging trial to evaluate the efficacy of adalimumab induction therapy in infliximab naïve CD patients. The primary endpoint was demonstration of a significant decrease in the rates of remission (CDAI < 150 points) at week 4 among the different dosed adalimumab groups and the placebo group. Rates of remission were 18% in the adalimumab 40 mg/20 mg group ($p = 0.36$), 24% in the 80 mg/40 mg group ($p = 0.06$), 36% in the 160 mg/80 mg group ($p = 0.001$) and 12% in the placebo group. Adalimumab was well tolerated.(75) Mian et al. described a 15-year-old girl with CD who developed an acute allergic reaction to infliximab infusion, despite premedication with solumedrol, diphenhydramine and acetaminophen.(76) She showed good clinical response to repeated adalimumab injections.Paediatric studies on the efficacy of adalimumab are not published yet. In CD patients who lost response, or developed infusion reactions or delayed-type hypersensitivity reactions to infliximab adalimumab may be well tolerated. Initially it was hypothesized that the amount of murine protein present in antibodies was related to

the immunogenicity, but it is now apparent that antibodies can also be induced to fully human therapeutic proteins, including adalimumab.(77) Since higher dosing of adalimumab -as compared to infliximab- seems necessary, further data on safety and costs are mandatory to outline the use in CD patients.

Etanercept (Enbrel) is a recombinant p75 TNF receptor/IgG fusion protein and is highly effective in treating rheumatoid arthritis and psoriasis.(78) However, in moderate-to-severe CD patients it is not effective.(79) Infliximab but not etanercept binds activated peripheral blood lymphocytes and induces apoptosis of peripheral and lamina propria lymphocytes, and this may be one explanation for the differences in efficacy of these two TNF-binding compounds.(7)

Certolizumab pegol (CDP870) is a pegylated anti-TNF antibody fragment for the treatment of CD. Schreiber et al. performed a randomized placebo-controlled trial in adult patients with moderate-to-severe CD.(80) The primary endpoint was the percentage of patients with decrease in CDAI score ≥ 100 points (clinical response) or CDAI ≤ 150 points (remission) at week 12. Two-hundred-ninety-two patients were treated with either 100 mg, 200 mg, 400 mg certolizumab pegol subcutaneously or placebo. Response rates were 36.5%, 36.1%, 44.4% and 35.6% respectively and therefore the difference was not statistically significant. However, post-hoc analysis demonstrated statistically significant increased certolizumab efficacy in the certolizumab 400 mg group. Recently, a randomized placebo-controlled multicentre study assessed efficacy and tolerability of 400 mg certolizumab pegol monthly in moderate-to-severe CD. Primary endpoints were decrease in CDAI of 100 points at weeks 6, and both at weeks 6 and 26 in patients with baseline CRP ≤ 10 mg/L. Response rates at weeks 6 and 26 of certolizumab pegol were 21.5% versus placebo 12.3% ($p<0.05$).(81) Treatment with this drug seems safe and well tolerated. Paediatric data are not available. Certolizumab pegol probably is a valuable new tool for the treatment of CD.

Conclusions and future research questions

Safety information on the use of infliximab in paediatric IBD patients is steadily increasing, but most information is collected retrospective in single centres treating small groups of paediatric CD patients over relatively short periods of time. Hence, there are no data on the long-term safety profile of infliximab in the paediatric setting, nor for long-term risks for development of malignancies and autoimmune disorders. It remains uncertain whether infliximab should be used episodically or as a scheduled maintenance therapy. Regularly scheduled maintenance therapy seems less immunogenic than episodic, but a significant number of patients initially treated with infliximab may remain in long-term remission. It is also not clear when long-term infliximab treatment should or can be discontinued, or whether early onset infliximab therapy will be disease-modifying in the longer term. These are all questions that should be answered by well-designed and long-term studies in paediatric CD patients. Research has to focus on all of these issues. Genetic, immunologic and clinical studies will help to further unravel the etiology of IBD. Subgroups of IBD patients will be classified with specific genotypes and phenotypes responding to particular treatment strategies and predict

positive response on infliximab. In the meanwhile, it is highly essential to continue safety monitoring in paediatric patients treated with infliximab. Most importantly, large, prospective multicentre randomized controlled studies have to be set up in paediatric IBD patients. For several reasons, results obtained with TNF binding compounds in adults cannot always be extrapolated to children. The clinical features of paediatric inflammatory bowel disease are different from the adult manifestations. For example in adults UC is predominantly confined to the rectum or left-sided colon while children usually present with pancolitis.(82,83,84) In adults diarrhoea (CD) or rectal bleeding (UC) is the most frequently presenting symptom while in children this is abdominal pain.(20) CD with paediatric-onset can cause considerable linear growth retardation.(85) Participation in normal childhood activities such as school and sports can be severely hampered. Metabolism may be different and early-onset disease may be more susceptible to infliximab. In view of the regulatory requirements that now include a full paediatric program pharmaceutical industry now has the obligation to design appropriate studies in children as a constitutive part of drug development. Proper evaluation of the efficacy and safety of infliximab in children will require the development of infrastructure and financial support to perform proper multicentre paediatric trials.

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Chapter 7

Infliximab as first line therapy in severe paediatric Crohn's disease

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Summary

A girl with severe Crohn's colitis is presented. She is the first child to be treated with infliximab and azathioprine as first line treatment (top-down versus step-up), and therefore corticosteroid naïve, with enormous success. The optimal treatment strategy concerning the use of infliximab in paediatric Crohn's disease patients is discussed.

Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease characterised by a transmural, granulomatous inflammation. Children with CD present with abdominal pain, (bloody) diarrhoea, fatigue and weight loss. Growth retardation and delayed pubertal development are often present and may be the first presentation. The current treatment strategy in paediatric CD consists of a step-up strategy. Aminosalicylates are started in mild disease, corticosteroids in moderate or severe disease. In case of insufficient response, additional immunomodulation (azathioprine, 6-mercaptopurine or methotrexate) is started. Since 1992, infliximab has been used as rescue medication as the conventional treatment failed.(1) We report a girl with severe Crohn's colitis who is the first child to be treated with infliximab and azathioprine as first line treatment (top-down versus step-up strategy).

Case report

An otherwise healthy 12-year-old girl presented with abdominal pain, diarrhoea, rectal bleeding for 3 months. She was very tired and was not able to attend school or walk long distances. During this period, she lost 4 kg of weight. On examination we saw a severely ill child, with tenderness in the right lower quadrant of the abdomen. There were no peritoneal signs like guarding or rebound tenderness, and there was no abdominal mass. Rectal examination revealed an empty ampulla and no further abnormalities. Length and growth were normal.
At first evaluation, laboratory investigations revealed erythrocyte sedimentation rate (ESR), 44 mm/h (normal < 20 mm/h); C-reactive protein, 224 mg/l (normal < 5 mg/l); albumin 28 g/l (normal 37.0-55.0 g/l), haemoglobin, 6.0 mmol/l (normal 7.5-9.8 mmol/l); leucocytes, 15.9 10^9/l (normal 4.6-13.5 10^9/l) with normal leukocyte differentiation and thrombocytes, 739

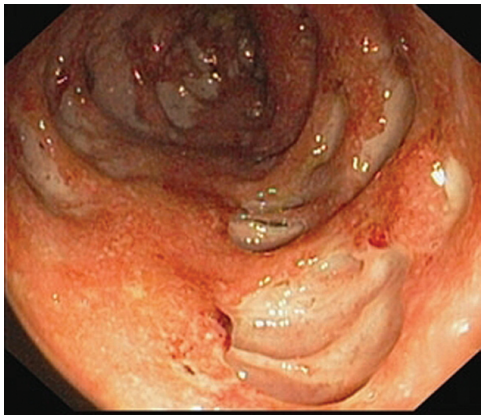

Figure 1 Colonic mucosa showing severe inflammation and multiple severe deep ulcerations.

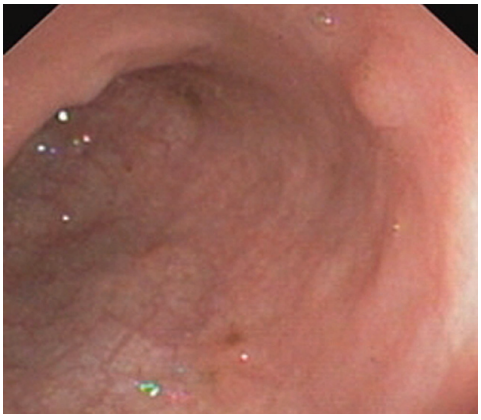

Figure 2 Completely healed colonic mucosa with normal vascular pattern and two pseudopolyps.

10^9/l (normal 150-350 10^9/l). Because of high suspicion of inflammatory bowel disease gastro- and colonoscopy were performed under general anaesthesia. Gastroduodenoscopy showed an unremarkable duodenum, some gastritis and several small aphthous lesions in the oesophagus. Ileocolonoscopy showed a normal terminal ileum throughout the whole colon however, severe inflammation and multiple severe deep ulcerations were seen (Crohn's Disease Endoscopic Index of Severity; CDEIS 24.0) (figure 1).(11) Enteroclysis showed signs of terminal ileitis. Histopathological examination revealed focal active histiocytic inflammation in the duodenal, gastric and oesophageal biopsies. Ileal biopsies revealed no abnormalities. Colonic biopsies revealed severe chronic, focal active inflammation with ulcerations. Severe Crohn's colitis was diagnosed. Paediatric Crohn's Disease Activity Index (PCDAI) at that point was 60.
Because of the severeness of the endoscopic pictures treatment was started according to a top-down instead of conventional step-up strategy; 3 infliximab infusions 5 mg/kg at week 0, 2 and 6 and azathioprine 2 mg/kg once daily. She did not receive any other medications, in particular no corticosteroids (apart from 2 single Di-adreson-F infusions, given immediately before the 2 first infliximab infusions, to prevent the formation of human anti-chimeric antibodies).

At second evaluation, 8 weeks after starting treatment, she was in clinical remission (PCDAI of 5). She went to school and she had no complaints of abdominal pain, diarrhoea or rectal bleeding. She had gained 3 kg of weight. Laboratory investigations revealed the following: ESR, 8 mm/h; C-reactive protein, < 1 mg/l; albumen, 44 g/l, haemoglobin, 6.8 mmol/l; leucocytes, 3.7 10^9/l with normal leukocyte differentiation and thrombocytes, 327 10^9/l.
Sigmoidoscopy was performed, and the colon was inspected up to the flexura lienalis. Completely healed mucosa was seen with a normal vascular pattern, as well as some pseudopolyps without any signs of inflammation (CDEIS 0.5) (figure 2).
Seven months after starting treatment, she was still in clinical remission (PCDAI of 5). Her only complaint was fatigue. Due to severe hair loss, she was switched from azathioprine to methotrexate, 10 mg weekly. Laboratory investigations revealed the following: ESR, 8 mm/h; C-reactive protein, < 1 mg/l; albumin, 44 g/l; haemoglobin, 8.1 mmol/l; leucocytes, 3.9 10^9/l with normal leukocyte differentiation; and thrombocytes, 264 10^9/l.

Discussion

An impressive clinical and endoscopic improvement was seen in a girl with severe Crohn's colitis after first line infliximab treatment combined with azathioprine. Apart from 2 single Di-adreson-F infusions, given immediately before the 2 first infliximab infusions, she did not receive any corticosteroid treatment.
Until now, infliximab is mainly used in patients with refractory CD, resistant to or dependent on corticosteroids, with or without immunomodulation (azathioprine, methotrexate). In 1992, in The Netherlands, the first patient with refractory CD (a 13-year-old girl) was successfully

treated with infliximab.(1) Over the past decade, infliximab is found to be effective and safe for the induction and maintenance of remission in adult patients with moderate to severe CD.(2) Clinical and endoscopic remission after infliximab treatment in refractory CD patients was first shown by Van Dullemen et al.(3) In children, non-controlled studies with infliximab reported similar results.(4,5) A trial in children suggested higher response rates to infliximab when the drug was initiated early in the disease course.(6) A recent randomized controlled trial in 130 adults with CD compared top-down treatment with 3 infusions of infliximab and azathioprine to step-up treatment with corticosteroids. A statistically higher effectivity of infliximab, if started according to the top-down strategy, in steroid-naïve patients, was shown.(7) This study in adults clearly demonstrates that it may be more effective to introduce infliximab early in the disease process instead of waiting until conventional therapy has failed. The early stages of immune-mediated disease may be more susceptible to immunomodulation. Recently, an abstract was published describing 10 paediatric CD patients receiving infliximab as initial therapy (in combination with azathioprine or methotrexate) versus 19 paediatric CD patients who were treated with solitary nutritional therapy and/or corticosteroids (in combination with azathioprine or methotrexate as maintenance therapy). Infliximab as first line therapy seemed to promote long term remission and seemed to change the disease course.(10)

In cases of moderate to severe paediatric CD, intensive treatment and adverse effects are unavoidable. Infliximab therapy is considered to be a safe therapy in the short term, but maintenance infliximab treatment is associated with the occurrence of infrequent but serious events, such as opportunistic infections or sepsis, allergic reactions and autoimmune disorders.(8) Another consideration is the earlier reported loss of response seen in almost half of the paediatric CD patients treated with maintenance infliximab.(5) However, steroid dependency is seen in 36%, and steroid resistance in 20% of the patients.(9) Furthermore, chronic use of corticosteroids has many well-known adverse effects such as growth retardation, osteoporosis, cataract, striae and facial swelling. Paediatric patients treated with infliximab as first line therapy might turn out to be dependent to infliximab. However, 6 infliximab infusions per year may be acceptable against the risk of a complicated course of CD with a high risk of developing perianal fistulas, repeated surgery and a marked decrease of the quality of life, as has been demonstrated in the step-up top-down study in adult patients with CD.(7) This large prospective, randomised trial demonstrated clear benefit of the step-up top-down strategy. A robust paediatric study is needed to confirm these findings.

The burning question whether to treat according to a step-up or top-down strategy remains to be answered. International collaboration between the continents is necessary to perform randomized controlled studies and to evaluate the optimal treatment therapeutic strategies of infliximab therapy in paediatric CD. Only then will the optimal long-term outcome in paediatric CD patients as concerns disease activity, number of relapses, hospital admissions, surgical intervention, growth and quality of life be answered.

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Chapter

8

Infliximab therapy in 30 patients with refractory paediatric Crohn's disease with and without fistulas in The Netherlands

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Summary

Objective

The purpose of this study was to describe the clinical experience with the anti-tumour necrosis factor chimeric monoclonal antibody, infliximab, in paediatric patients with Crohn's disease in The Netherlands.

Design

Descriptive.

Methods

Clinical response and adverse effects of infliximab were recorded for paediatric patients with Crohn's disease treated from October 1992 to January 2003.

Results

Thirty patients (aged 7-18 years) with refractory Crohn's disease (with or without severe fistulas) were treated with infliximab. Patients were treated with up to 30 infusions. Mean follow-up was 25.3 months. A total of 212 infusions were administered. Thirteen patients had refractory Crohn's disease without fistulas. Six patients showed good long-term response to infliximab treatment (defined as clinical index $\leq$ 10 points). Sixteen patients had refractory Crohn's disease with draining fistulas. Nine showed good long-term response (closure or nonproductiveness of fistulas). One patient with metastatic Crohn's disease in the skin had a good long-term response. Six patients developed an allergic reaction during infusion. In one patient, the allergic reaction occurred after an infliximab-free interval of 9 years. One patient died of sepsis.

Conclusions

Infliximab was an effective therapy in 53% of patients with refractory paediatric Crohn's disease, with or without fistulas. Approximately half of the patients became unresponsive to infliximab therapy. Randomized controlled studies are mandatory to assess long-term efficacy and safety to define the optimal therapeutic strategy of infliximab therapy in children with Crohn's disease.

Introduction

Standard medical treatment of mild Crohn's disease consists of aminosalicylates. In moderate and severe disease, corticosteroid is the treatment of choice. Steroid dependency is seen in 36% and steroid resistance is seen in 20% of Crohn's disease patients.(1) Furthermore, long-term use of corticosteroids has many well-known adverse effects.(2) The next step in therapy is immunomodulation with drugs such as azathioprine, 6-mercaptopurine or methotrexate. These drugs have a delayed onset of action and can cause serious adverse effects (pancreatitis, hepatitis and severe leucopoenia).

A recent development in refractory Crohn's disease (steroid dependent and/or therapy resistant despite immunomodulators) is the use of the monoclonal antibody anti-tumour necrosis factor-α infliximab. Tumour necrosis factor α has a key role in the inflammation cascade. Its concentration in the intestinal mucosa is increased in children with active Crohn's disease.(3) In The Netherlands, in 1992, the first patient with refractory Crohn's disease (a 13-year-old girl) was successfully treated with infliximab.(4) In the past decade, infliximab has been shown to be effective and safe for the induction and maintenance of remission in adult patients with moderate to severe Crohn's disease with or without fistulas.(5-10) In children, noncontrolled studies of infliximab have produced similar results.(11-15) The aim of this study is to describe the experience with infliximab in all paediatric patients with severe Crohn's disease in The Netherlands.

Materials and methods

A study group of the Dutch paediatric gastroenterological society composed a consensus guideline for infliximab treatment of children with severe Crohn's disease (table 1). Subsequently, all paediatric gastroenterology units in The Netherlands were solicited to report data on infliximab-treated children and adolescents with Crohn's disease. The participating hospitals were visited, and data on patient characteristics, disease history, previous treatment, surgery, complications, and effect of infliximab treatment were extracted from the medical record. Data collection was closed on January 1st, 2003.

A modified Paediatric Crohn's disease Activity Index (PCDAI) (16,17) was calculated before the first infusion of infliximab and 4 weeks after the first infusion (initial response). In patients receiving infliximab maintenance therapy, the modified PCDAI was calculated 4 weeks after the last administered infusion. Clinical response was defined as good if the index was $\leq$10 or showed a decline of $\geq$ 20 points. In fistulous disease, response was considered good if fistula closure or cessation of drainage was maintained for $\geq$ 4 weeks by physical examination.

Table 1. Consensus guideline of Dutch paediatric gastro-enterologists for infliximab therapy in children / adolescents with Crohn's disease

Inclusion criteria

- active therapy resistant Crohn's disease (≥30 points according to the "paediatric Crohn's disease activity index" or ≥200 points according to the "Crohn's disease activity index"), despite treatment with corticosteroids (1-2 mg/kg; minimal duration 3 weeks) and azathioprine (2-2.5 mg/kg; minimal duration 4 months)
- perianal fistulas, despite treatment with metronidazol (15-20 mg/kg; minimal duration 4 weeks) and azathioprine (2-2.5 mg/kg; minimal duration 6 weeks) or cyclosporine (2.5-6 mg/kg; minimal duration 4 weeks)
- steroid toxicity (contra-indication to steroid treatment due to side effects) or steroid dependence (development of flare-up of clinical symptoms, each time steroids are withdrawn)

Exclusion criteria

- renal insufficiency (upper limit 3 x normal value creatinine for age)
- elevated transaminases (upper limit 3 x normal values for age)
- (suspicion of) tuberculosis
- leucopenia (<3 x 10^9/l)
- anaemia (haemoglobin <4.5 mmol/l)
- (wish for) pregnancy

Medication

- refractory Crohn's disease: infliximab 5 mg/kg intravenously in 2 hours, every 8 weeks for 1 year, continue in case of good response
- severe fistulas (after magnetic resonance imaging and drainage of abscess if present): infliximab 5 mg/kg intravenously in 2 hours, week 0, 2 and 6; continue if flare-up of clinical symptoms occurs
- in case no effect is seen (anymore): discuss whether to give a higher dose or to shorten the interval

Results

Between October 1st, 1992, and January 1st, 2003, 30 patients (15 girls, 15 boys) were treated in 7 hospitals according to the treatment guidelines. Thirteen had refractory Crohn's disease (without fistulas), 16 had active disease with fistulas and 1 had metastatic Crohn's disease (table 2). The mean age at diagnosis was 11.4 years (range, 2.7-16.8 years). All patients were refractory to drug treatment and had been treated with 5-aminosalicylic acids, high-dose corticosteroids for 3 weeks or more and azathioprine for 4 months or more without success, or they had become intolerant to the above-mentioned drugs.

The mean age at the start of infliximab therapy was 14.1 years (range, 7.2-18.2 years). At the start of treatment, 20 patients (67%) had had Crohn's disease for ≥ 2 years. The mean duration of follow-up was 25.3 months (range, 4-122 months). During the infliximab treatment, 28 patients also used azathioprine or methotrexate and 18 patients used corticosteroids (table 2). In total, 212 infliximab infusions were administered, 1-30 per patient (mean, 7.1).

Patients with refractory Crohn's disease without fistulas

Thirteen patients had active Crohn's disease despite high-dose corticosteroids and/or azathioprine administration. These refractory patients had a mean Modified PCDAI of 29.2 (range, 15-45) before the first infusion of infliximab. Four weeks after the first infusion,

mean Modified PCDAI was 9.2 (range, 0-35). Twelve patients (92%) showed good initial response (Modified PCDAI of ≤ 10 or a decline of ≥ 20 points 4 weeks after first infusion). Six patients (46%) had sustained good response to infliximab therapy after a mean period of 20 months (fig. 1). Two patients (patients 2 and 8) achieved stable clinical remission and infliximab therapy was discontinued (table 2). Patient 8, diagnosed 11 months before his first infliximab infusion, achieved prolonged remission after one infusion. As in all of the other patients, the patient had first been treated with 5-aminosalicylic acids and corticosteroids. When that failed, he received azathioprine. He received infliximab because remission was still not achieved. The fact that infliximab treatment was given early in the disease course may explain the prolonged remission achieved after only one infusion. The other 4 patients with sustained good response (patients 1, 3, 11 and 12) are still receiving infliximab therapy with Modified PCDAI scores of 0-5, four weeks after the last infusion. Infusions are given every 8 weeks unless there is evidence of disease exacerbation, then the interval is shortened to sustain remission.

Six of the 13 refractory patients had growth impairment (a change of at least -1.0 SD for height). After the start of infliximab treatment, 3 patients (2, 3 and 12) resumed normal linear growth velocity. They are still receiving maintenance infliximab treatment. The other

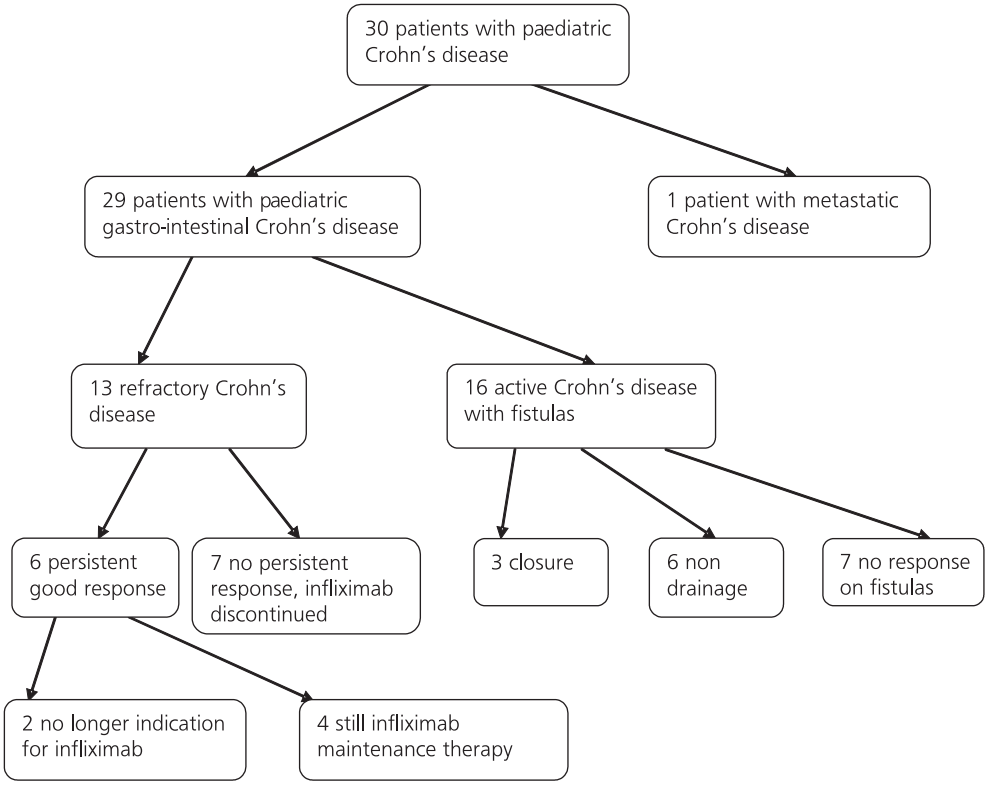

Figure 1. Flow record demonstrating outcomes of patients with paediatric Crohn's disease treated with infliximab.

Table 2. Patient characteristics and infliximab therapy refractory Crohn's disease

nr	age at start ifx (yrs)	gender	location; complications	total of gifts
1	9.2	girl	pancolitis	18 (every 6 weeks)
2	18.3	boy	ileum, coecum, colon; growth retardation	10
3	16.5	boy	ileum, colon; growth retardation	7
4	7.1	boy	colon; steroid dependent, growth retardation	2
5	14.8	girl	colon; steroid dependent	8
6	15.8	boy	colon; arthritis	6
7	17.8	girl	colon	4
8	13.6	boy	colon	1
9	8.9	girl	colon, final diagnosis colitis ulcerosa	2
10	9.8	girl	ileum and colon; growth retardation	4
11	11	boy	pancolitis	5 (every 5 weeks)
12	15.8	boy	pancolitis; growth retardation, steroid dependent	8
13	15	girl	ileum, pancolitis; growth retardation, steroid dependent	4
fistulas				
14	15.1	girl	perianal fistulas	23 (every 4 weeks)
15	14.8	girl	perianal and recto-vaginal fistulas	6 (last gift 10 mg/kg)
16	11.5	boy	abdominal wall fistulas	5
17	17.5	girl	recto-vaginal fistulas	30 (every 4-6 weeks)
18	15.5	girl	recto-vaginal fistulas	14
19	12.1	girl	perianal fistulas and abscesses	3
20	15	girl	perianal fistulas	4
21	15.7	boy	perianal fistulas and abscesses	8
22	16.3	boy	perianal fistula	4
23	10.2	boy	perianal fistulas	9
24	17.4	girl	perianal fistulas and abscesses	3
25	16.6	girl	recto-vaginal fistula	3
26	14.6	boy	perianal fistula	4
27	14.3	girl	perianal fistula	3

	efficacy	outcome	FU (months)	concomitant medication
	good response	still ifx	26	methotrexate
	good response and growth recovery	ifx stopped, remission	30	azathioprine
	good response, withdrawal of steroids possible, growth recovery	still ifx	20	azathioprine
	short response after first infusion, no response after 2nd infusion	ifx stopped, subtotal colectomy	14	steroids, methotrexate switched to azathioprine
	initially good response, finally no response any longer	ifx stopped, subtotal colectomy	48	steroids, azathioprine switched to methotrexate
	initially good response, finally no response any longer	ifx stopped, colectomy planned	50	steroids
	good response after first infusion, very short response after 2nd infusion	ifx stopped, colectomy	35	steroids, azathioprine
	remission ever since	ifx stopped, remission	32	azathioprine
	good response after first infusion, short response after 2nd infusion	ifx stopped, subtotal colectomy	12	azathioprine
	short response after first infusion, no response after consequent infusions	ifx stopped	28	steroids, azathioprine
	good response	still ifx	6	steroids, azathioprine
	initially good response, then diminished response, growth recovery	still ifx	8	steroids, azathioprine switched to methotrexate
	initially good response, stopped because of allergy	ifx stopped, colectomy	122	steroids, azathioprine
	ileostomy because of uncontrollable exacerbations, no drainage	still ifx	28	azathioprine, temporary switch to methotrexate
	initially complete remission and closure fistulas, finally no response	ifx stopped, subtotal colectomy	23	azathioprine, temporary switch to methotrexate
	little response	died due to sepsis and MOF	5	steroids, azathioprine
	little disease activity, fistulas no response	still ifx	50	steroids, azathioprine
	no drainage	still ifx	29	azathioprine
	no response	ifx stopped	18	steroids, azathioprine
	no response	fistula surgery planned	10	steroids, azathioprine
	initially closure fistulas, after that exacerbation, stop ifx, colectomy	ifx stopped, colectomy	16	steroids, azathioprine
	almost complete closure fistula	ifx stopped, remission	11	steroids, azathioprine
	no effect on fistulas, little disease activity	still ifx	37	steroids, azathioprine
	persistent closure fistulas	ifx stopped	36	non
	no drainage	ifx stopped	7	azathioprine
	no drainage, growth recovery	ifx stopped	4	steroids, azathioprine
	initially no drainage, then exacerbation under treatment	still ifx	4	azathioprine

Table 2 continued.

nr	age at start ifx (yrs)	gender	location; complications	total of gifts
28	17	boy	perianal fistula	3
29	16.7	boy	perianal fistula	3
metastatic Crohn's disease				
30	10.9	boy	skin penis and scrotum	8

FU: follow-up; ifx: infliximab

three patients did not resume normal linear growth velocity. Two patients (4 and 10) became unresponsive to infliximab and the treatment was discontinued. The third patient (13) was the first patient to be treated with infliximab. She was treated with two consecutive infusions, and maintenance therapy was not considered at that time.(4)

Seven of the 13 refractory patients had no clinical response to repeated infliximab infusions and therapy was discontinued. Five of these 7 patients underwent total or subtotal colectomy and in one patient, colectomy is planned.

Patients with active Crohn's disease and fistulas

Sixteen patients had active Crohn's disease with fistulas – 11 with perianal fistulas only, 3 with rectovaginal fistulas, 1 with both perianal and rectovaginal fistulas and 1 with abdominal wall fistulas. Continued draining fistulas despite antibiotics and immunomodulatory treatment were the indication for infliximab treatment.

Nine of 16 patients (56%) had good clinical response to infliximab therapy (fig. 1). In two patients (24 and 29), fistulas closed after three infliximab infusions. In the first patient, the fistulas are still closed after follow-up of 36 months. In the second patient, a new fistula developed 9 months after infliximab therapy was discontinued. The fistulas of 7 patients (14, 18, 22 and 25-28) stopped draining after infliximab therapy. In 2 patients (17 and 23) fistulas did not close or cease draining; however, the Crohn's disease activity diminished and infliximab treatment was continued (modified PCDAI not available).

Five patients were unresponsive to therapy. Two patients (15 and 21) underwent colectomy after infliximab failure. In 1 patient (20), surgical correction of the fistulas is planned. Another patient (16) was an 11-year-old boy with refractory colonic Crohn's disease (modified PCDAI 55 before infliximab therapy). He had extensive abdominal wall fistulas and had undergone several resections, stoma revisions and fistula correction before he came to The Netherlands for treatment. He was treated for several months with total parenteral nutrition, corticosteroids, azathioprine and repeated surgical intervention before infliximab was started. Five months later, uncontrollable bacterial sepsis developed with multiorgan failure and the patient died. The sepsis may have originated from an abscess located near a stenosis in the colon. The patient was leucopoenic, probably because of long-standing azathioprine therapy.

	efficacy	outcome	FU (months)	concomitant medication
	initially no drainage, exacerbation after stop ifx	ifx stopped	8	steroids, azathioprine
	closure fistula, after 9 months again a peri-anal fistula	ifx stopped	16	steroids, azathioprine
	good response; finally skin resection	ifx stopped, remission	25	azathioprine

Patient with metastatic Crohn's disease

Patient 30 had metastatic Crohn's disease in the skin of his penis and scrotum with granulomas identified on biopsy of the affected skin. There was no sign of disease in the gastrointestinal tract.(18) His oedema and tissue inflammation diminished after a single infliximab infusion. The patient received repeated infusions for a year followed by surgery.

Entire cohort

Analysis of response of the entire cohort according to total of infliximab infusions received was also performed. The patients showing response to infliximab therapy received 1 to 30 infusions (fig. 2). The patients who did not show response to infliximab therapy received 2 to 8 infusions (fig. 3).

Adverse effects of infliximab therapy

Six of the 30 patients (20%) had an immediate allergic reaction during infusion. One of the patients received only corticosteroids as concomitant therapy. The other 5 patients received azathioprine (3 with simultaneous corticosteroids) as concomitant therapy. Corticosteroids

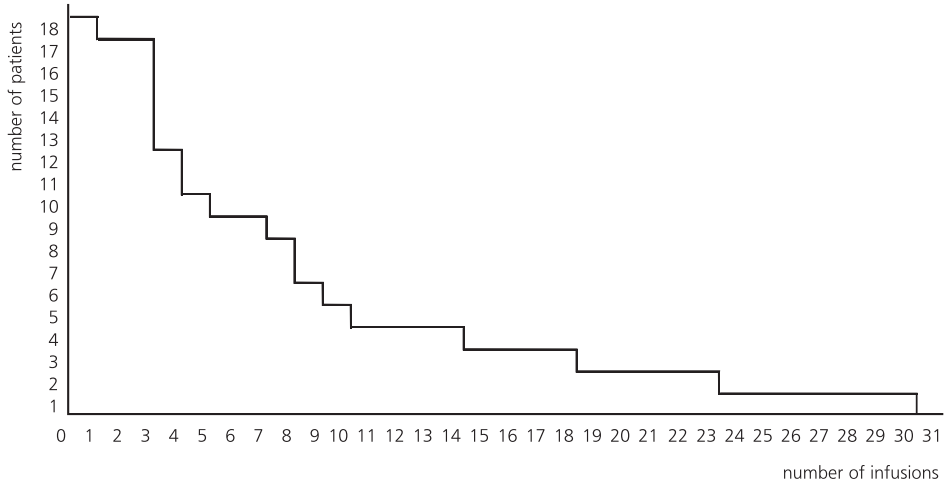

Figure 2: Patients responding to infliximab therapy related to total amount of infliximab infusions received

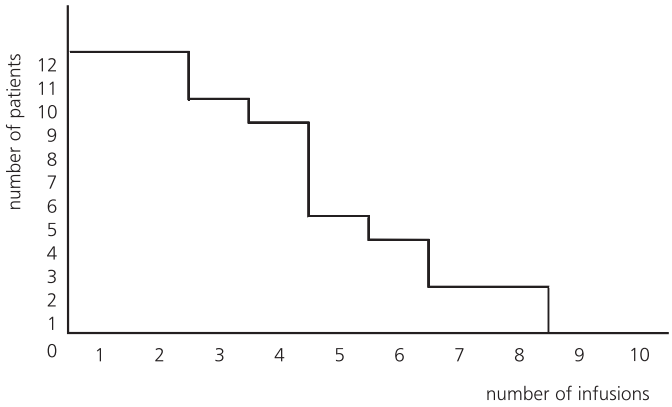

Figure 3: Patients not responding to infliximab therapy related to total amount of infliximab infusions received

and an antihistamine were administered intravenously, after which the reactions resolved. These medications were given as prophylaxis before subsequent infusions. One patient (13) experienced a severe allergic reaction with dyspnoea, facial oedema and hypoxemia after the fourth infliximab infusion that improved after treatment with corticosteroids, antihistamines and epinephrine intravenously. Nine years had elapsed between her second and third infusions. She received no further infliximab therapy. Antibodies to infliximab were not routinely checked in our patients.

Seven patients (23%) experienced other adverse effects. Six reported headache, muscle ache and nausea after infusions. One boy (patient 23) developed arthritis of the hip joint after the second infliximab infusion. It is unclear whether this adverse event was caused by infliximab. Neither of the patients experienced autoimmune phenomena.

Discussion

This study describes the experience with infliximab therapy in a cohort of Dutch children and adolescents with refractory Crohn's disease. This is the longest followed paediatric cohort receiving repeated infusions of infliximab. After a mean follow-up of 2 years, 53% of the patients have continued good clinical response to infliximab therapy. These results are comparable to other recently reported cohorts of paediatric and adult patients with Crohn's disease.(10,14)

Because the PCDAI (15) is difficult to apply in daily practice and requires laboratory examinations, we used a modified PCDAI, which was recently validated and proven to be as accurate as the PCDAI.(16) In the modified PCDAI, only clinical parameters are evaluated in order to reduce discomfort for the patient. The cut-off score for remission is 15 according to the PCDAI (19) and 10 according to the modified PCDAI.

We observed a good clinical response as shown by regained normal linear growth velocity in 3 of 6 patients with growth retardation after initiation of infliximab treatment. Ineffectively controlled inflammation with subsequent chronic malnutrition is an important factor for growth impairment in children with Crohn's disease.(20) Conversely, normal growth is a marker of treatment success.

In our study, the response of patients with fistulas to infliximab therapy was scored as closure or nondrainage. The fistulas were not routinely imaged on follow-up. However, infliximab treatment does not induce disappearance of fistulous tracts, irrespective of therapeutic response.(21)

Nonresponsiveness to infliximab occurred in 47% of our patients. Our results suggest that patients who remain in remission on infliximab after receiving 9 infusions will remain responsive to the medication. It is of great importance to find clinical, biochemical, immunologic or genetic markers to predict nonresponsiveness. Early reactivation of the inflammatory cascade, higher serum tumour necrosis factor-α levels and antibody formation are influencing factors (22-24). All but 2 patients (6 and 24) received concomitant immunomodulation. The formation of human antichimeric antibodies was not routinely checked. Therefore, it is not possible to determine whether the absence of concomitant immunomodulation or the formation of human antichimeric antibodies are factors associated with nonresponsiveness to infliximab.

Allergic reactions during infliximab infusion occurred in 20% of our patients but resulted in discontinuation of therapy in only one patient who experienced a severe systemic reaction after a long infliximab-free interval. The development of systemic reactions after an infliximab-free interval has not been previously reported in a paediatric patient.(25) Another adverse effect of infliximab therapy is the increased risk for infections. In our study, one boy died of sepsis after treatment with infliximab. This patient was malnourished, had undergone multiple surgical procedures, and was leucopoenic as a consequence of azathioprine therapy. Nonetheless, severe infections have been previously reported in infliximab-treated patients, and they should be diagnosed early and treated promptly. Until now, infliximab therapy has been considered safe in the short term. However, it is clear from our data that maintenance infliximab treatment is associated with a risk for severe infections and a relatively high rate of allergic reactions.

The mean follow-up of our patients was 2 years (maximum, 10 years). No malignancies were seen. However, this is much too short a time to conclude that infliximab is a safe drug in the long term.

The majority of our patients had had Crohn's disease for more than 2 years when infliximab treatment was started and all were resistant to conventional therapy. The optimal dosing scheme for infliximab treatment is still unknown. Concomitant immunosuppressive therapy seems to be associated with decreased immunogenicity and fewer adverse effects.(26) It may be more effective to introduce infliximab earlier in the disease process instead of waiting until all conventional therapy has failed since the early stages of this condition may be more susceptible to immunomodulation.(11) Moreover, debilitating side effects of corticosteroids

may be avoided while quality of life is improved. Our results suggest that infliximab maintenance be given at intervals of 6-8 weeks.

Infliximab is an effective therapy in approximately half of the patients with refractory paediatric Crohn's disease with or without fistulas. Approximately half of our patients, however, became nonresponsive to infliximab therapy. In children with Crohn's disease, randomized controlled studies are mandatory to further assess short and long-term safety, responsiveness and optimal therapeutic strategies of infliximab therapy.

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Chapter 9

Infliximab in paediatric Crohn's disease: long-term follow-up of an unselected cohort

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Summary

Objective

The authors previously described the efficacy and safety of infliximab maintenance therapy in Dutch children with Crohn's disease (CD). The aim of this study was to describe 3 more years' experience in children and adolescents with active CD treated with infliximab.

Methods

Clinical response and adverse events were recorded for all Dutch paediatric CD patients treated with infliximab from October 1992 to June 2006.

Results

Sixty-two CD patients (36 boys) in 9 hospitals were treated with infliximab. Mean age at the start of infliximab therapy was 14.2 years (range, 7.1-18.2 years). Mean follow-up since the start of infliximab was 32 months. In total, 744 infliximab infusions were administered. Analysis of the entire cohort demonstrates that 14.5% of patients had prolonged response, while 59.7% were infliximab dependent and 22.6% lost response. In total, 8 patients (12.9%) developed an infection during infliximab therapy and 7 of the 62 patients (11.3%) had an immediate allergic reaction during infusion.

Conclusions

Clinical response to infliximab therapy was seen in 75% of patients. However, 60% of the patients in this cohort are dependent on repeated infliximab infusions. Infliximab maintenance therapy seems very effective and safe in paediatric CD. Long-term safety however, is still of major concern.

Introduction

Infliximab (Remicade®) is a chimeric monoclonal antibody (75% human, 25% murine) that avidly binds human tumor necrosis factor-α (TNF-α), a cytokine with a central role in the pathogenesis of many inflammatory processes.(1) Large randomized controlled trials have shown efficacy and safety of infliximab for the induction and maintenance of remission in adult patients with active Crohn's disease (CD).(2-5) Following these clinical studies infliximab was approved by the U.S. Food and Drug Administration (FDA) in 1998 for short-term treatment of moderately to severely active luminal and fistulizing CD in adults and, approximately 5 years later, for maintenance therapy. In children and adolescents mostly small, non-randomized and non-placebo controlled studies support the notion that infliximab is a very potent drug in a population that is not responding to standard therapies. It is ironical that, although the first patient treated with infliximab, 16 years ago, was a 13-year-old child, infliximab only very recently has been approved for treatment of moderate to severely active paediatric CD.(6,7)
The safety of infliximab is of major concern, and the most frequently encountered severe adverse events are related to severe infections and re-activation of tuberculosis. The association of infliximab with the development of cancer or lymphomas remains unclear. Non-life threatening infusion reactions do occur rather frequently and seem to be related to formation of antibodies to infliximab. It has been suggested that it may be more effective to use infliximab early in the disease process instead of waiting until conventional therapy has failed. The early stages of immune-mediated disease may be more susceptible to immunomodulation and the natural history of CD may be altered.(8,9) In children prolonged duration of response after infliximab therapy is reported when the drug was initiated early in the disease course.(10) Since there are so many unanswered questions concerning infliximab therapy in children with CD, it is of importance to learn from the past. We earlier reported on the efficacy and safety of infliximab therapy in Dutch children with CD.(11) The aim of the present study was to describe 3 more years' experience with infliximab in a larger group of children and adolescents with active CD, with or without fistulas.

Methods

In 2001 the Dutch paediatric gastroenterological society developed consensus guidelines for infliximab treatment of children with moderate-to-severe CD, resistant to conventional therapy.(11) These guidelines are described in chapter 8, table 1, page 102. Subsequently, all paediatric gastroenterology units in The Netherlands were solicited yearly to report data on paediatric CD patients treated with infliximab. Patients were included in case infliximab was started before the age of 19 years. Data on patient characteristics, disease history, previous treatment, adverse events, number and schedule of infliximab infusions and surgery were extracted from the medical records. Data collection was closed on the 1st of June 2006.

The outcome of infliximab therapy was defined as prolonged, infliximab dependency, lost response or no response. These definitions are further clarified in table 2. In the previous study a modified Paediatric Crohn Disease Activity Index (PCDAI) was calculated at set time points.(11) In this study however, it was no longer possible to score patients at set time points since different schedules for patient visits were used and patients sometimes had long intervals between these visits.

Statistical analysis was performed using SPSS. Prolonged response was compared to no prolonged response in fistulizing and non-fistulizing patients using $\chi 2$ and Fisher's exact tests. The response to infliximab was analyzed in patients starting infliximab within or after 1 and 2 years since diagnosis using $\chi 2$ and Fisher's exact tests. A p value of <0.05 was considered to be significant.

Results

Between October 1st, 1992, and June 1st, 2006, 62 CD patients (36 boys) were treated with infliximab in 9 hospitals (7 tertiary and 2 secondary centres) (table 3). A total of 29 patients were already reported in the previous study (one patient from the previous study was excluded in the present study since the diagnosis CD was switched to UC).(11) In the present study 33 patients were added. Mean age at diagnosis was 12 years (range 2.8-16.8 years). All patients were refractory to conventional treatment consisting of either sustained enteral nutrition, 5-aminosalicylic acid or high-dose corticosteroids for a minimum of 3 weeks as remission induction. As maintenance therapy they were treated with either adequately dosed azathioprine or methotrexate for a minimum of 4 months (unless adverse events to these drugs prohibited further usage). One patient presented with such severe clinical and endoscopic colonic CD that first-line infliximab therapy was administered.(12) Mean age at the start of infliximab therapy was 14.2 years (range, 7.1-18.2 years) (table 3). Mean follow-up since the start of infliximab was 32 months (range 2-165 months). Ten patients (16.7%) were lost to follow-up after a mean period of 28.6 months (range 11-50) due to transfer to an adult gastroenterologist after their 18th birthday. In total, 744 infliximab infusions were administered; 1-64 per patient (mean 12.0).

Analysis of the entire cohort demonstrates that 14.5% of patients had prolonged response, while 59.7% were infliximab dependent and 22.6% lost response.(Table 4) Selective analysis of the 33 patients added in the present study revealed that 4 (12.1%) patients had prolonged response, while 28 (84.8%) were infliximab dependent and 1 patient (3.0%) lost response.

Out of the 47 patients who received at least 5 infliximab infusions, 9 patients (19.1%) stepped up to 10 mg/kg infliximab because of decrease in response and in 20 patients (42.6%) the interval was shortened to 4-7 weeks because of loss of effect before the end of the 8 week interval. Twenty-two out of 62 patients (35.5%) underwent surgery, after infliximab was started. Sixteen patients were operated because of no or lost response to infliximab. Five

patients were dependent on infliximab infusions and underwent surgery because of stenosis (n=1), abscess (n=2) or intercurrent exacerbation (n=2). One patient initially had prolonged response, infliximab was discontinued. Six months later the patient developed an exacerbation and underwent partial ileal resection. There was no statistical difference regarding response to infliximab therapy in the entire cohort when started within 1 or within 2 years after CD was diagnosed as compared to after 1 or after 2 years (table 5).

A total of 45 patients had refractory luminal CD without fistulas. Of these patients one patient (2%) had prolonged response while 31 patients (68.9%) were dependent on infliximab infusions (table 5). Sixteen patients had fistulizing CD. In this group 8 patients (50.0%) had prolonged response and 5 patients (31.1%) were dependent on infliximab infusions to maintain response. Therefore, prolonged response occurred significantly more often in patients with fistulizing disease compared to those CD children without fistulas ($p<0.001$).

One patient (a 13-year-old girl) presented with a very severe Crohn's colitis and therefore was treated with first line infliximab therapy (5 mg/kg, induction at week 0, 2, 6 followed by maintenance every 8 weeks). Azathioprine was started at the same time. Eight weeks after starting treatment, she was in complete clinical and endoscopic remission. Twelve months later she had had a total of 9 infusions and was in remission ever since.(12)

Another patient was treated with infliximab because of an extraintestinal manifestation, being metastatic CD in the skin of his penis and scrotum with granulomas identified on biopsy of the affected skin, without any intestinal manifestation of the disease.(13) He received 8 infliximab infusions and attained complete remission. Interestingly, 2 years after infliximab therapy was stopped, he developed Crohn's colitis, while he had no gastrointestinal manifestation before. His extraintestinal manifestation did not flare. This time, azathioprine maintenance was started and because of intolerance subsequently switched to methotrexate. After this switch complete remission was attained.

Adverse events

In total, 8 patients (12.9%) developed an infection during infliximab therapy (table 6).

An 11-year-old boy treated with infliximab had a fatal outcome following septicemia. This patient already is described in our previous study.(11) He was the only patient who suffered a severe infection. Seven patients had mild infections. A 14-year-old boy developed multiple stenoses of the ileum after the third infliximab infusion and had to be operated on.

Infusion reactions

Seven out of the 62 patients (11.3%) had an immediate allergic reaction during infusion (table 6). One of these patients received corticosteroids as the only concomitant medication; all other patients received azathioprine as concomitant medication for periods of 3 months or more. In all but one patient reaction resolved after administration of intravenous corticosteroids and an antihistamine. In one patient the allergic reaction was so severe that epinephrine was administered intravenously. The allergic reaction followed an infliximab-free interval of 9 years.(11) Prophylaxis (such as corticosteroids and antihistamine) was not given

before restarting infliximab. Infliximab therapy was discontinued. In 2 more patients infliximab was discontinued because of allergic reactions. A 17-year-old girl had an allergic reaction with her 20th infliximab infusion, 26 months after the first one. Intravenous corticosteroids and antihistamine were given as prophylaxis before subsequent infusions. However, at the same time she lost response which did not return after stepping up to 10 mg/kg infliximab. Therefore, infliximab therapy was stopped. A 15-year-old boy had an infliximab-free interval of 13 months between his third and fourth infusion and developed an allergic reaction on the fourth infusion. Despite corticosteroids and antihistamine prophylaxis, allergic reactions re-occurred during treatment and therefore infliximab therapy was stopped. Antibodies to infliximab were not routinely checked in our patients.

Discussion

This study describes the longest follow-up of a large paediatric CD cohort receiving repeated infusions of infliximab. This cohort is unselected and therefore mirrors daily practice. In our study, sustained clinical response to maintenance infliximab therapy was seen in 75% of patients, with a mean follow-up of 32 months. Sixty percent of this cohort is dependent on repeated infliximab infusions. Prolonged response occurred significantly more often in fistulizing CD since 50% of the children with fistulizing CD had prolonged response as against 2% of children with non-fistulizing CD ($p<0.001$). Rutgeerts et al. already demonstrated in adult CD patients that after one single infusion, approximately 80% of responders relapsed within one year unless re-treatment was provided.(13) In 1999 2 large, international placebo-controlled, randomized double-blind trials, ACCENT I (573 adult patients with moderate to severe luminal CD) and ACCENT 2 (306 adult CD patients with one or more draining fistulas of at least three months duration) evaluated infliximab maintenance therapy for a period of 54 weeks. These trials illustrated that adult CD patients with luminal disease responding to an initial dose of infliximab are more likely to be in remission at weeks 30 and 54, to discontinue corticosteroids, and to maintain response for a longer time, when infliximab treatment is continued every 8 weeks.(4) Fistulizing CD patients receiving infliximab maintenance therapy had significantly longer median time to loss of response compared to placebo (> 40 weeks vs.14 weeks, $p<0.001$).(5) Present et al. demonstrated in a randomized placebo-controlled trial that 55% of adult patients with fistulas receiving infliximab 5 mg/kg had complete closure for 1 month.(3)

In children and adolescents mostly small, non-randomized and non-placebo controlled studies have been performed. Recently a randomized, multicentre, open-label study (REACH) was conducted evaluating the safety and efficacy of infliximab maintenance treatment in moderate to severe paediatric CD patients aged 6 to 17 (n=112). Responding patients were randomized at week 10 to receive infliximab every 8 weeks (n=52) or every 12 weeks (n=51) through week 46. Response to infliximab was almost 90% at week 10. At 54 weeks,

64% of patients (33 of 52) receiving infliximab maintenance every 8 weeks were in clinical response as assessed by a paediatric Crohn's disease activity index (decrease from baseline ≥15 and ≤30 total), compared with 33% (17 of 51) in the every-12-week maintenance group ($p<0.001$).(14) These data suggest that the effects of infliximab treatment of children may be better than the results in adult patients, both for remission induction and for maintenance therapy. Wewer et al. demonstrated that out of a total of 24 paediatric CD patients (median age 15.4 years) 29% had a prolonged response after discontinuation of infliximab therapy, 42% was dependent on infliximab reinfusion, while 29% was unresponsive to infliximab. Follow-up was 90 days after intended cessation of infliximab therapy.(15)

After analysis of only the patients that were included after 2002 in this study, it is striking to conclude that 96.9% of patients had clinical response to infliximab therapy. This success percentage is both higher than the results of the REACH study and than the 53% response rate from our previous study.(11,14) Probably, the increasing clinical experience with infliximab therapy has lowered the threshold for initiating infliximab therapy, which presently may be started in less severely diseased patients. This hypothesis is supported by the amount of patients being enrolled, being 29 patients in a period of 5 years in the previous study (excluding the girl who was already treated in 1992) and 33 patients in a period of 3.5 years thereafter. The difference between the present and our previous study cannot be explained by a shorter follow-up, since the opposite is the case (32 months as against 25 months).

Increasing clinical experience and data from randomized controlled trials (although mainly performed in adult patients) also will have improved the knowledge on optimal infliximab therapy. CD patients with indication for infliximab now routinely start with induction at week 0, 2 and 6 and continue infliximab as maintenance therapy in case of response, while they are on adequate immunomodulation. Another explanation is that many patients in our study cohort received increased dosage and/or shortened intervals between infliximab infusions. In the REACH study, intervals less than 8 weeks were not permitted.

The results of our study may seem less robust than those of the REACH study, as clinical response in our study is defined by the treating physicians. However, we believe that by using the response definitions as mentioned earlier, clinical efficacy of infliximab is reflected sufficiently.

In an open-label, prospective clinical trial Kugathasan et al. found that the therapeutic effect of infliximab was sustained longer in paediatric patients with early CD (disease duration less than 2 years) compared to patients with long-standing CD (more than 2 years).(10) In their retrospective study Lionetti et al. found that children diagnosed with CD less than 1 year before had a higher chance on a longer duration of response than children known with CD for more than 1 year.(16) In contrast to these findings we were not able to show a difference in response between early and late disease for more or less than both 1 and 2 years interval between diagnosis and start of infliximab (table 5).

The main adverse events in our patient cohort were infections and infusion reactions.

Infections were seen in 12.9% of our patients, but in only one (1.6%) of these patients it concerned a severe infection (unfortunately being fatal). Pooling of several paediatric studies

shows that serious adverse events were described in 4.7% (26 out of 548) of patients, being Listeria monocytogenes meningitis (n=1), serious or unusual infections such as abscess, reactivation of Epstein Barr virus and shingles (n=22) and intestinal strictures (n=3).(14-23). In adult CD patients 2 large studies on the safety of infliximab are conducted.

Colombel et al. investigated the safety profile in 500 adult CD patients treated with 2211 infusions in total. In 6.0% of patients severe adverse events possibly related to infliximab infusions were seen. These adverse events contained severe infections in 15 patients. Ten patients died, 5 out of these 10 (0.8%) were judged as likely or possibly related to infliximab. One of these patients had a contra-indication to infliximab (a persistent abdominal abscess), 6 of the 10 had severe co morbidity associated with CD.(24) The interpretation of the relationship with infliximab treatment is difficult, because most of these patients were severely ill and were also treated with corticosteroids and immunomodulatory drugs. The TREAT registry is a prospective, observational, multicentre, long-term registry including 6290 CD patients with nearly 15,000 patient-years of follow-up evaluating pre-specified safety-related outcomes. Half of the patients received infliximab, whereas half received other therapies, such as corticosteroids and immunomodulators. Although unadjusted analysis showed an increased risk for infection while on infliximab therapy, multivariate logistic regression analysis suggested that infliximab was not an independent risk factor of serious infections. The incidence of malignancies in the 2 groups was similar, as was the incidence of lymphoma.(25,26)

Numbers of children with CD treated with infliximab are still much too small and follow-up still much too short to be properly informed about the long-term safety profile. This is illustrated by the recent post-marketing surveillance data which prompted the manufacturer of infliximab, Centocor inc., to report the occurrence of 8 cases of hepatosplenic T-cell lymphoma (6 out of 8 with a fatal outcome) in young CD patients out of approximately 270,000 CD patients treated with infliximab. Allergic reactions during infliximab therapy occurred in 11.3% of our patients which is comparable to the 3 cohort studies determining the rate of infusion reactions in 57 children receiving 361 infliximab infusions, 111 children receiving 594 infliximab infusions and 243 children receiving 1652 infliximab infusions respectively.(23,27,28) Infusion reactions occurred in 8.5%, 8.1% and 16.5% respectively of patients and in 9.7%, 1.5% and 3.6% respectively of all infusions.

Interestingly, 2 patients developed an allergic reaction after a prolonged infliximab free interval. From the ACCENT I trial we know that antibodies to infliximab were detected in 30% in the episodic-treated patients compared with 8% in the maintenance-treated patients.(4) It remains uncertain whether infliximab should be used episodically or as a scheduled maintenance therapy. Regularly scheduled maintenance therapy seems less immunogenic than episodic therapy, but especially patients with primary fistulizing CD patients initially treated with infliximab may remain in long-term remission.

Ten patients were lost to follow-up in this study. Mean follow-up in these 10 patients was approximately 30 months, therefore the efficacy of infliximab treatment in these patients is well known. However, information on long-term adverse events is lacking.

In conclusion, infliximab is a very effective therapy in a population that is not responding to standard therapies. This study shows a clinical response to infliximab therapy in 75% of patients. It is important to note, however, that 60% of this cohort is dependent on repeated infliximab infusions. In patients with fistulizing disease prolonged response to infliximab therapy was seen in 50%. Based on these findings and other paediatric and adult studies performed hitherto, the best therapeutic strategy for paediatric patients with refractory luminal or fistulizing CD seems to be maintenance infliximab therapy. When the patient achieves complete remission it may be worthwhile to stop infliximab infusions. Achievement of complete remission can be confirmed by endoscopic evaluation. In case of exacerbation infliximab maintenance therapy can in most cases safely be restarted. Surgery must be considered for localized disease. In patients with fistulizing disease, infliximab can be discontinued after achieving complete remission. In case of re-occurrence of fistulas infliximab can be restarted.

Advantages of maintenance infliximab therapy in moderate-to-severe paediatric CD seem to outweigh the disadvantages. However, thorough evaluation of the efficacy and safety of infliximab and of the optimal therapeutic strategy in children is still mandatory. This will require the development of infrastructure and financial support to perform prospective treatment registration in a multi-center database as well as proper multicenter paediatric trials.

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Chapter 10

Summary

Summary

In this thesis paediatric-onset inflammatory bowel disease (IBD) is evaluated. IBD is a debilitating disease having an enormous impact upon the lives of IBD patients. This impact is even bigger in children and adolescents. Their physical, social and emotional functioning can be comprised and their growth, psychomotor and intellectual development hampered. However, after growing up, they should have the opportunity to have a social life, found a family and get a job. As yet, IBD cannot be cured. It is a challenge to optimize diagnostics and therapeutics and thereby minimize disease activity. Disease exacerbations, complications of treatment, growth retardation and surgery must be avoided. Optimal development in all ways should be achieved.
This thesis is dedicated to these goals. To be able to understand the aetiology of paediatric IBD genetics are explored. To further improve diagnostics endoscopy is studied. The therapeutics azathioprine and infliximab are thoroughly studied since azathioprine is widely used as maintenance treatment in paediatric IBD patients and infliximab is a recent development in the treatment of refractory Crohn's disease (CD). Both drugs are evaluated to improve effectiveness and reduce side-effects in paediatric IBD patients.

In **chapter 2** genetic susceptibility in paediatric IBD is studied. This may have a more important role in the aetiology of early-, than of late-onset IBD. If so, a higher frequency of the gene mutations can be expected in paediatric IBD patients. The purpose of our study was to determine the frequency of NOD2/CARD15, TLR 4, OCTN and DLG5 mutations in paediatric-onset IBD, compare these data with adult-onset IBD and to identify genotype-phenotype associations. NOD2/CARD15 3020Cins and OCTN rs3792876 mutations occurred statistically significant more often in paediatric-onset compared to adult-onset CD. Therefore, some susceptibility gene polymorphisms indeed occur more often in paediatric-onset CD.

In **chapter 3** the diagnostic yield of our endoscopic policy (pancolonoscopy together with esophagogastroduodenoscopy in case of accompanying complaints such as abdominal pain or diarrhoea) in children presenting with prolonged rectal bleeding is evaluated.
Over an 8-year period at the Emma Children's Hospital/Academic Medical Centre 147 colonoscopies were performed in 137 paediatric patients (63 boys). In 80% of patients a diagnosis was established after the first colonoscopy. IBD (33.6%) and polyp(s) (18.2%) were the most prevalent diagnoses, respectively. In 72% of patients diagnosed as CD, focal, chronically active gastritis was seen on histology, giving support to the diagnosis CD. In 22% of patients with polyps, polyps would have been missed in case only sigmoidoscopy was performed. No complications were seen. Therefore, pancolonoscopy is the investigation of choice in children with prolonged rectal bleeding. In patients presenting with accompanying complaints it is advisable to perform ileocolonoscopy combined with esophagogastroduodenoscopy. This combines a high diagnostic yield with a safe procedure.

In **chapter 4** we describe a study on pharmacogenetics of azathioprine in a paediatric IBD cohort.
Azathioprine is an important maintenance drug in paediatric IBD. The occurrence and type of adverse events to azathioprine may be related to thiopurine S-methyltransferase (TPMT) enzyme activity and to Inosine Triphophate Pyrophosphatase (ITPase) deficiency. Seventy-two azathioprine treated paediatric IBD patients were assessed for *TPMT* and *ITPA* polymorphisms and for adverse events. Eleven patients experienced an adverse event for which azathioprine was stopped: pancreatitis (n=4), leucopoenia (n=2) and "general malaise" (n=5). Of the 11 patients who stopped azathioprine due to adverse events, 10 had wild type alleles for all investigated genotypes. Genotyping of *ITPA 94C>A* polymorphisms showed that two patients were homozygous, both tolerated azathioprine well. In children who do not tolerate this drug, we were not able to demonstrate an association with polymorphisms in the *ITPA* gene and *TPMT* polymorphisms. Other causes for azathioprine intolerance remain to be elucidated.

In **chapter 5** the use of azathioprine, a prodrug of 6-Mercaptopurine, used for maintenance of remission of CD in Europe is studied. We evaluated to what extent azathioprine is used in newly diagnosed paediatric CD patients and whether maintenance of remission differed between patients using azathioprine or not. Eighty-eight children were included of which 72 (82%) received azathioprine during the follow-up period (38±17 months). Patients diagnosed after 2000 received azathioprine significantly earlier during the course of disease, compared to those diagnosed earlier. Median maintenance of first remission in patients who initially used corticosteroids was longer in patients receiving azathioprine, compared with non-azathioprine patients. Azathioprine patients had longer corticosteroid free periods than non-azathioprine patients (AZA patients 575 days vs. non-AZA patients, 259 days; $p<0.05$). The use of azathioprine is associated with prolonged maintenance of first remission in paediatric CD patients.

In **chapter 6** infliximab use in children and adolescents with IBD is reviewed. Large randomized controlled trials have shown efficacy and safety of infliximab for the induction and maintenance of remission in adult patients with active CD. In children and adolescents mostly small, non-randomized and non-placebo controlled studies supported the notion that infliximab is a very potent drug in a population that is not responding to standard therapies. The safety of infliximab is of major concern, and the most frequent severe adverse events are related to severe infections and re-activation of tuberculosis. The association of infliximab with development of cancer or lymphomas remains unclear. Non-life threatening infusion reactions do occur rather frequently and seem to be related to formation of antibodies to infliximab. There are indications that the early stages of CD might be more susceptible to immunomodulation and the natural history of CD might be altered by introduction of infliximab early in the disease process instead of waiting until conventional therapy has failed.

In **chapter 7** a girl with severe Crohn's colitis is presented. She is the first child to be treated with infliximab and azathioprine as first line treatment (top-down versus step-up), and therefore corticosteroid naïve. An impressive clinical and endoscopic improvement was seen 8 weeks after the first infliximab infusion and 7 months after starting treatment, she was still in clinical remission.
Until now, inflixmab is mainly used in patients with refractory CD, resistant to or dependent on corticosteroids, with or without immunomodulation (azathioprine, methotrexate). The burning question whether to treat according to a step-up or top-down strategy remains to be answered.

In **chapter 8** the clinical experience with infliximab treatment in paediatric patients with CD in The Netherlands is described. Thirty patients (aged 7-18 years) with refractory Crohn's disease (with or without severe fistulas) were treated with infliximab. Patients were treated with up to 30 infusions, with a mean follow-up of 25.3 months. In total 212 infusions were administered. Thirteen patients had refractory CD without fistulas. Six patients showed good long-term response on infliximab treatment (defined as clinical index ≤ 10 points). Sixteen patients had refractory CD with draining fistulas. Nine of these showed good long-term response (closure or non-productiveness of fistulas). One patient had metastatic CD in the skin and showed good long-term response. Of the 30 patients, 6 patients developed an allergic reaction during infusion. In one patient this occurred after an infliximab free interval of 9 years. One patient died due to a sepsis. Infliximab was an effective therapy in 53% of patients with refractory paediatric CD, with or without fistulas. Approximately half of the patients however became non-responsive to infliximab therapy.

In **chapter 9** 3 more years' experience with 3 times as much infliximab infusions in twice as much children and adolescents with active CD, with or without fistulas is described.
By now, 62 CD patients (36 boys) in 9 hospitals were treated with infliximab. Mean follow-up since the start of infliximab was 32 months (range 2-165 months). In total, 744 infliximab infusions were administered; 1-64 per patient (mean 12.0). Analysis of the entire cohort demonstrated that 14.5% of patients had prolonged response, while 59.7% were infliximab dependent and 22.6% lost response. Sixteen patients had fistulising CD, 8 of these patients (50.0%) had prolonged response. In total, 8 patients (12.9%) developed an infection during infliximab therapy and 7 of the 62 patients (11.3%) had an immediate allergic reaction during infusion. Infliximab maintenance therapy seems very effective and safe in refractory paediatric CD.

Discussion and future perspectives

In this thesis is demonstrated that genetic susceptibility has a more important role in the aetiology of early-, than of late-onset IBD. Besides, genotype-phenotype relations are described. These findings are important since this provides more and more insight into disease pathogenesis. In future, genetic susceptibility research in large paediatric-onset IBD cohorts will discover new involved polymorphisms and the influence of these mutations on disease behaviour will be established. Ultimately, this will lead to improved treatment strategies.

Evaluation of endoscopy in children presenting with rectal bleeding revealed that pancolonoscopy including intubation of the ileum together with upper endoscopy is indicated. Otherwise inconclusive diagnosis may lead to repeated examinations and/or suboptimal treatment. Since localization and severity of CD determine both therapy and prognosis the whole gastrointestinal tract, including the small bowel, has to be examined in the diagnostic work-up. Presently, a clinical trial on Magnetic Resonance Imaging (MRI) versus complete endoscopy and enteroclysis as diagnostic work-up in children and adolescents suspected for IBD is ongoing (beyond the scope of this thesis). In future, enteroclysis probably will be replaced by MRI. Though endoscopy and histopathology will maintain an important role in diagnosing IBD, MRI might also replace endoscopy in the follow-up of the disease, since MRI is less invasive and allows transmural evaluation of the gastrointestinal tract.

Azathioprine is widely used as maintenance therapy in paediatric IBD. The corticosteroid sparing effect of azathioprine is of major importance in the treatment of children. However, its well-known toxicity such as bone marrow suppression, hepatitis and pancreatitis clearly limits prescription of this drug. The occurrence and type of adverse events to azathioprine might be related to thiopurine S-methyltransferase (TPMT) enzyme activity and to Inosine Triphophate Pyrophosphatase (ITPase) deficiency and therefore to functional *ITPA* and *TPMT* polymorphisms. However, no association of functional *ITPA* and *TPMT* polymorphisms and the occurrence of azathioprine related adverse events could be detected. Presently, pharmacogenetic assessment prior to thiopurine therapy seems not warranted. In future new genes and enzymes influencing azathioprine metabolism will be found. Consequently, therapeutic drug monitoring (genotyping and metabolite measurements) will prevent most adverse events and provide optimal efficacy of azathioprine treatment.

Evaluation of 14 years of Dutch experience with infliximab therapy in paediatric IBD demonstrates that infliximab is a very effective therapy in a population not responding to conventional therapies, including azathioprine treatment. Safety in the short-term is quite acceptable, but the recent occurrence of 8 cases of hepatosplenic T-cell lymphoma in young CD patients treated with both azathioprine and infliximab is very worrisome. This illustrates the necessity of performing prospective trials on every new therapy used in children. Safety of such therapy decades later is a major concern.

In future, genotyping will tell which patients develop moderate to severe CD and will have good clinical response on infliximab. These children will be treated with first line infliximab therapy. No corticosteroids will be administered any longer, thereby avoiding steroid toxicity.

Future IBD research should be directed at new immunobiologic therapies with the ultimate goal of curing or even better preventing the disease. Will it be possible to reset the immunologic system and eliminate the abnormal reactivity against luminal and mucosal antigens? Or will gene therapy be the ultimate solution, after all aetiology of IBD partly is genetic? Maybe, in high risk patients, environmental factors can be thus influenced that abnormal reactivity of the immune system will not occur, hereby preventing the occurrence of IBD. It will be exciting to follow all IBD developments worldwide.

Nederlandse samenvatting

Nederlandse Samenvatting

In dit proefschrift worden chronische ontstekingen van de darm (in het Engels *inflammatory bowel disease*; IBD) op de kinderleeftijd bestudeerd. De twee belangrijkste vormen van chronische darmontsteking zijn de ziekte van Crohn en colitis ulcerosa.
De ziekte van Crohn is een chronische ontsteking van de gehele darmwand die verspreid door het gehele maagdarm traject (oftewel 'van mond tot kont') kan voorkomen. Colitis ulcerosa is een chronische ontsteking van het slijmvlies van de dikke darm. IBD is een invaliderende ziekte en heeft een grote invloed op het dagelijkse leven van een IBD-patiënt. Deze invloed is nog groter als de patiënt een kind of adolescent is. Hun lichamelijke conditie en sociale en emotionele welbevinden kunnen beperkt zijn en hun groei, geestelijk en motorisch functioneren en intellectuele ontwikkeling kunnen ernstige vertraging oplopen. Toch moeten ook deze patiënten, eenmaal op volwassen leeftijd, de kans hebben op een goed ontwikkeld sociaal leven, een gezinsleven en een goede baan.
Vooralsnog is IBD niet te genezen. Het is een uitdaging zowel diagnostiek als therapie dusdanig te optimaliseren dat de ziekteactiviteit zo gering mogelijk is. Ziekte-exacerbaties, complicaties van behandeling, groeiachterstand en chirurgisch ingrijpen dienen zoveel mogelijk vermeden te worden. Er moet niet alleen gestreefd worden naar optimale ontwikkeling op het lichamelijke vlak, maar ook op het sociale en maatschappelijke vlak. Dit proefschrift is opgedragen aan deze doelstellingen. Om de ontstaanswijze van IBD op de kinderleeftijd beter te begrijpen, is de genetische achtergrond van kinderen met IBD nader onderzocht. Om de diagnostiek verder te verbeteren, is endoscopie op de kinderleeftijd bestudeerd. De medicijnen azathioprine en infliximab zijn grondig bestudeerd; azathioprine wordt veel gebruikt als onderhoudstherapie bij kinderen met IBD en infliximab is een nieuw medicijn voor de behandeling van de ziekte van Crohn. Hierbij is gezocht naar methoden om de toepassing van beide medicijnen bij kinderen met IBD nog effectiever en veiliger te maken.

In **hoofdstuk 2** is de genetische (erfelijke) aanleg voor IBD bij kinderen met deze aandoening bestudeerd. Het kan zijn dat genetische aanleg een grotere rol speelt in het ontstaan van IBD op de kinderleeftijd dan op volwassen leeftijd. Uit eerdere studies zijn varianten van genen bekend die de kans om IBD te krijgen vergroten. Deze varianten zijn o.a. *NOD2/CARD15*, *TLR4*, *OCTN* en *DLG5*. Als deze genetische aanleg inderdaad een grotere rol speelt bij kinderen, is er een hogere frequentie van genmutaties bij kinderen met IBD te verwachten dan bij volwassenen met IBD. Het doel van deze studie was om de frequentie van mutaties in *NOD2/CARD15*, *TLR4*, *OCTN* en *DLG5* te bepalen bij IBD begonnen op de kinderleeftijd. De uitkomsten werden vergeleken met de mutatiefrequenties bij IBD begonnen op de volwassen leeftijd. De mutaties *NOD2/CARD15* 3020Cins en *OCTN* rs3792876 bleken statistisch significant vaker voor te komen bij patiënten bij wie ziekte van Crohn op kinderleeftijd begonnen was dan bij patiënten bij wie de ziekte van Crohn op volwassen leeftijd begonnen was. Bepaalde genvarianten die de kans op het ontstaan van ziekte van Crohn vergroten,

blijken dus inderdaad vaker voor te komen bij patiënten bij wie de aandoening op de kinderleeftijd ontstaan is.

In **hoofdstuk 3** is de diagnostische waarde geëvalueerd van een totale coloscopie bij kinderen die worden gepresenteerd met persisterend rectaal bloedverlies (gecombineerd met endoscopie van het ileum en slokdarm, maag en duodenum als er sprake was van bijkomende klachten, zoals buikpijn en diarree).
Gedurende een periode van 8 jaar zijn in het Emma Kinderziekenhuis/AMC 147 coloscopiën verricht bij 137 kinderen (63 jongens). Bij 80% van de patiënten werd een diagnose gesteld na de eerste coloscopie. IBD (33,6%) en poliepen (18,2%) waren de meest voorkomende diagnosen. Bij 72% van de patiënten die ziekte van Crohn bleken te hebben, werd focale, chronisch actieve gastritis gezien bij histologie, wat de diagnose ziekte van Crohn ondersteunde.
Bij 22% van de patiënten met poliepen zouden poliepen gemist zijn als endoscopie beperkt was gebleven tot sigmoïdoscopie. Er traden geen complicaties op als gevolg van de endoscopie. Geconcludeerd werd dat endoscopie van de gehele dikke darm het aangewezen onderzoek is bij kinderen die zich presenteren met persisterend rectaal bloedverlies. Indien deze kinderen bijkomende klachten hebben, is het aan te bevelen eveneens endoscopie van het ileum, slokdarm, maag en duodenum te verrichten. Dit beleid combineert een hoge diagnostische opbrengst met een hoge veiligheid.

In **hoofdstuk 4** wordt een farmacogenetische studie van azathioprine in een pediatrisch IBD-cohort beschreven.
Azathioprine speelt een belangrijke rol bij de medicamenteuze onderhoudstherapie van kinderen met IBD. Het optreden van bijwerkingen van azathioprinegebruik is mogelijk gerelateerd aan de enzymactiviteit van thiopurine-S-methyltransferase (TPMT) en aan inosinetrifosfaatpyrofosfatase (ITPase)-deficiëntie. Van 72 kinderen met IBD, behandeld met azathioprine, werden *TPMT*- and *ITPA*-polymorfismen en bijwerkingen in kaart gebracht. Bij 11 patiënten werd de behandeling met azathioprine gestaakt vanwege bijwerkingen: pancreatitis (n=4), leukopenie (n=2) en algemene malaise (n=5). Tien van deze 11 patiënten hadden normale allelen van alle onderzochte genotypen. Uit genotypering van *ITPA 94C>A* bleek dat 2 patiënten homozygoot waren voor de mutatie. Beiden verdroegen azathioprine echter goed. Hieruit werd geconcludeerd dat het niet verdragen van azathioprine niet geassocieerd was met de aanwezigheid van mutaties in het *ITPA*- of *TPMT*-gen. verder onderzoek naar intolerantie voor azathioprine dient zich te richten op andere oorzaken.

In **hoofdstuk 5** wordt een onderzoek naar het gebruik van azathioprine voor het behoud van remissie bij de ziekte van Crohn bij kinderen besschreven. Onderzocht werd hoeveel kinderen met ziekte van Crohn behandeld werden met azathioprine en of behoud van remissie vaker voorkwam bij kinderen die azathioprine gebruikten. Er werden 88 kinderen geïncludeerd, van wie er 72 (82%) behandeld waren met azathioprine ten tijde van de follow-up periode

(38±17 maanden). Bij patiënten bij wie na 2000 de diagnose ziekte van Crohn was gesteld, werd significant eerder azathioprine gestart dan bij patiënten die al voor 2000 met deze aandoening bekend waren. De mediane duur van behoud van eerste remissie bij patiënten die in eerste instantie behandeld werden met corticosteroïden, was langer bij de patiënten die behandeld werden met azathioprine (re-activatie van de ziekteactiviteit: 544 [met azathioprine] versus 254 dagen [zonder azathioprine], p=0,08; herstart corticosteroïden: 575 [met azathioprine] versus 259 dagen [zonder azathioprine], p<0,05). Geconcludeerd kan worden dat azathioprine sinds 2000 eerder wordt geïntroduceerd in de behandeling van nieuw gediagnosticeerde ziekte van Crohn bij kinderen. Azathioprinegebruik is geassocieerd met langduriger behoud van eerste remissie bij kinderen met ziekte van Crohn.

Hoofdstuk 6 is een uitgebreid overzicht van de bestaande literatuur over infliximabgebruik bij kinderen en adolescenten met IBD. Grote gerandomiseerde en gecontroleerde trials hebben aangetoond dat infliximab als inductie- en onderhoudsbehandeling bij volwassen patiënten met actieve ziekte van Crohn effectief en veilig is. Bij kinderen en adolescenten zijn vooral kleine, niet-gerandomiseerde en niet placebogecontroleerde studies verricht. Deze studies ondersteunen de bevinding dat infliximab een zeer effectief middel is in een populatie die niet reageert op de standaardtherapie. Daarbij is het uiteraard van groot belang of het middel ook veilig kan worden toegepast. Het blijkt dat als bijwerking vooral ernstige infecties (waaronder re-activering van tuberculose) gerapporteerd zijn. Mogelijk is er een associatie tussen infliximabgebruik en het ontstaan van kanker en in het bijzonder lymfomen. Niet-levensbedreigende infusiereacties treden relatief frequent op en lijken gerelateerd de zijn aan antistofvorming tegen infliximab. Er zijn aanwijzingen dat een kind in het vroege stadium van ziekte van Crohn gevoeliger is voor immunomodulatie en dat het natuurlijke beloop van de ziekte van Crohn anders kan verlopen door in een vroeg stadium infliximab te starten in plaats van hiermee pas te beginnen als de conventionele therapie gefaald heeft.

In **hoofdstuk 7** wordt een meisje met ernstige Crohnse colitis beschreven. Zij is het eerste kind bij wie als eerstelijnsbehandeling de combinatie infliximab en azathioprine is gegeven (*top-down*) en dus niet is begonnen met corticosteroïden (*step-up*). Acht weken na de eerste infliximabinfusie werd een indrukwekkende klinische en endoscopische verbetering gezien en 7 maanden na aanvang van de behandeling was zij nog steeds in klinische remissie.
Tot nu toe is infliximab alleen voorgeschreven aan patiënten met refractaire ziekte van Crohn, resistent voor of afhankelijk van corticosteroïdbehandeling, veelal in combinatie met immunomodulatie (azathioprine of methotrexaat). De belangrijke vraag welke behandeling het best is, *top-down* of *step-up*, moet nog beantwoord worden.

In **hoofdstuk 8** wordt de klinische ervaring beschreven met infliximabbehandeling bij kinderen met ziekte van Crohn in Nederland. Dertig patiënten met refractaire ziekte van Crohn (leeftijd 7-18 jaar, met en zonder fistels) werden behandeld met infliximab. Patiënten kregen 1-30 infusies en de gemiddelde follow-up was 25,3 maanden. In totaal werden 212

infusies toegediend. Dertien patiënten hadden refractaire ziekte van Crohn zonder fistels. Zes patiënten vertoonden een goede langetermijnrespons op de infliximabbehandeling (gedefinieerd als klinische index ≤10 punten). Zestien patiënten hadden refractaire CD met actieve fistels. Negen van deze 16 vertoonden een goede lange termijn respons (sluiting of inactiviteit van fistels). Eén patiënt had metastatische ziekte van Crohn in de huid, hij vertoonde eveneens een goede lange termijn respons. Zes van de 30 patiënten ontwikkelden een allergische reactie tijdens de infusie. Bij één patiënt trad deze reactie op na een infliximabvrij interval van 9 jaar. Eén patiënt overleed ten gevolge van een sepsis. Infliximab bleek een effectieve therapie bij 53% van de kinderen met refractaire ziekte van Crohn. Ongeveer de helft van hen reageerde op de langere termijn echter niet meer op infliximabbehandeling.

In **hoofdstuk 9** is de ervaring met infliximabbehandeling beschreven gedurende een nog langere tijd en in een nog groter cohort kinderen met actieve ziekte van Crohn (met en zonder fistels).
Ten tijde van dit onderzoek waren 62 kinderen (36 jongens) met ziekte van Crohn in 9 ziekenhuizen behandeld met infliximab. De gemiddelde follow-up vanaf de start van de behandeling was 32 maanden (spreiding 2-165 maanden). In totaal werden 744 infliximabinfusies toegediend; 1-64 per patiënt (gemiddeld 12). Analyse van het gehele cohort liet zien dat 14,5% van de patiënten een langdurige respons vertoonde na het staken van de infusies, terwijl 59,7% afhankelijk was van herhaalde infliximabinfusies en 22,6% de respons verloor ondanks herhaalde infusies. Van de 16 patiënten met ziekte van Crohn met fistelvorming vertoonden 8 patiënten (50%) een langdurige respons op de infliximabinfusies. Bij acht patiënten (12,9%) trad ten tijde van de behandeling met infliximab een infectie op en bij 7 patiënten (11,3%) ontstond een acute allergische reactie tijdens de infusie. Onderhoudstherapie met infliximab lijkt een zeer effectieve en voldoende veilige behandeling te zijn bij kinderen met refractaire ziekte van Crohn.

Discussie en toekomstverwachtingen

In dit proefschrift wordt beschreven dat genetische vatbaarheid een grotere rol speelt in de etiologie van IBD wanneer deze ontstaan is op de kinderleeftijd. Daarbij worden genotype-fenotype-associaties gevonden. Zulke bevindingen zijn van belang, daar ze meer inzicht verschaffen in de etiologie van IBD. In de toekomst zal genetisch onderzoek bij grote kinder-IBD-cohorten nog meer betrokken polymorfismen kunnen aantonen en de invloed van deze mutaties op het ziektegedrag zal steeds duidelijker worden. Uiteindelijk kan dit leiden tot verbeterde behandelingsstrategieën.

Evaluatie van endoscopie bij kinderen met rectaal bloedverlies liet zien dat endoscopie van het gehele colon (inclusief endoscopie van het ileum en de bovenste tractus digestivus indien er sprake is van co-morbiditeit) geïndiceerd is bij deze categorie patiënten. Onvolledige diagnostiek leidt tot een onvolledige diagnose, met als risico dat (belastende) onderzoeken herhaald moeten worden of dat de behandeling suboptimaal is. Bij de ziekte van Crohn zijn de prognose en de behandeling afhankelijk van zowel lokalisatie (uitgebreidheid) als ernst van de ontsteking. Daarom is het essentieel bij diagnosestelling het gehele maagdarmkanaal (inclusief dunne darm) te onderzoeken op ziekteactiviteit. Recent is een klinische trial aangaande kernspinresonantie (MRI) versus complete endoscopie en enteroclyse als onderzoek bij diagnosestelling bij kinderen en adolescenten met verdenking op IBD afgesloten (deze valt buiten het bestek van dit proefschrift). In de toekomst wordt de klassieke enteroclyse waarschijnlijk vervangen door MRI-enteroclyse. Hoewel endoscopie en histologie een belangrijke rol zullen houden bij de diagnostiek van IBD, is die rol mogelijk weggelegd voor MRI bij het vervolgen van de ziekteactiviteit. MRI is immers minder invasief en verschaft tevens een transmuraal beeld van het maagdarmkanaal.

Azathioprine is een onderhoudsmedicijn dat op grote schaal wordt voorgeschreven aan kinderen met IBD. Het corticosteroïdsparende effect van azathioprine is van groot belang bij de behandeling van kinderen. Helaas kan azathioprinegebruik leiden tot beenmergsuppressie, hepatitis en pancreatitis. Het optreden van deze bijwerkingen zou kunnen samenhangen met de thiopurine-S-methyltransferase (TPMT)-enzymactiviteit of de aanwezigheid van inosinetrifosfaatpyrofosfatase (ITPase)-deficiëntie of aan functionele *TPMT*- en *ITPA*-polymorfismen. Er kon echter geen associatie gevonden worden tussen functionele polymorfismen van *TPMT* en *ITPA* en het optreden van bijwerkingen van azathioprinegebruik. Op dit moment is routinematige farmacogenetische bepaling voorafgaand aan behandeling met azathioprine dan ook niet geïndiceerd. In de toekomst kan van nieuwe genen en enzymen worden vastgesteld dat ze een rol spelen in het azathioprinemetabolisme. Uiteindelijk kan wellicht zogeheten *therapeutic drug monitoring*, op basis van genotypering en concentratiebepalingen van van metabolieten, de meeste bijwerkingen voorkomen en zorg dragen voor een zo hoog mogelijke effectiviteit van azathioprine.

Evaluatie van 14 jaar ervaring met infliximab in Nederland bij IBD op de kinderleeftijd demonstreert dat infliximab een zeer effectieve therapie is bij kinderen die niet reageren op conventionele therapie, inclusief behandeling met azathioprine. De veiligheid op korte termijn

is zeer acceptabel. Recent zijn echter jonge crohnpatiënten beschreven die behandeld werden met azathioprine en infliximab en bij wie een hepatosplenisch T-cellymfoom ontstond. De veiligheid op lange termijn is dus reden tot grote zorg. Dit illustreert eveneens de specifieke noodzaak van prospectieve trials bij kinderen naar de effecten van alle nieuwe medicamenten die bedoeld zijn voor gebruik in de kindergeneeskunde. In de toekomst kan met genotypering worden vastgesteld welke patiënten matig tot ernstige ziekte van Crohn ontwikkelen en welke een goede respons op infliximabtherapie zullen vertonen. Deze kinderen kunnen dan in aanmerking komen voor eerstelijnstherapie met infliximab. Corticosteroïden hoeven dan niet langer voorgeschreven te worden, waardoor corticosteroïdtoxiciteit vermeden wordt.
Toekomstig IBD-onderzoek dient gericht te zijn op nieuwe immunologische therapieën met als ultiem doel genezing of zelfs preventie van IBD. Zal het mogelijk zijn het immunologische systeem te herprogrammeren en de abnormale hyperreactiviteit tegen luminale en mucosale antigenen te elimineren? Of zal gentherapie de ultieme oplossing zijn, aangezien de etiologie van IBD deels genetisch bepaald is? Misschien kunnen de omgevingsfactoren bij hoogrisicopatiënten dusdanig beïnvloed worden dat de abnormale reactie van het immuunsysteem niet optreedt, waardoor het ontstaan van IBD voorkomen wordt. Het zal spannend zijn alle ontwikkelingen op IBD-gebied wereldwijd te volgen.

Dankwoord

Dankwoord

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En zonder wie dit alles niets...
Marcel, Lara en Amber Brouwer

Curriculum vitae

Lissy de Ridder werd op 28 mei 1969 geboren te Uitgeest. In 1988 deed zij eindexamen aan het Murmellius Gymnasium te Alkmaar. Uitloting voor de studie geneeskunde volgde, waarna zij 1 jaar au-pair werd bij een Zweeds gezin in Gävle. Het jaar daarop werd zij alsnog ingeloot. Zij volgde haar studie Geneeskunde aan de UvA te Amsterdam van 1988 tot 1995. Als onderdeel van de studie verbleef ze in 1991 4 maanden te India en werd daar "Medical Officer in Leprosy".
Omdat het grote doel was kinderarts te worden, volgde er een tijd van agnio-schappen door het hele land (Dordrecht, Nijmegen, Den Bosch en Amsterdam) waarna de opleiding in het Bosch MediCentrum (thans Jeroen Bosch ziekenhuis) en in het Emma Kinderziekenhuis/AMC volgde. In april 2003 werd haar opleiding tot kinderarts afgerond.
In mei 2003 startte haar fellowship kindergastro-enterologie in het Emma Kinderziekenhuis/AMC onder leiding van Jan Taminiau en Marc Benninga. Onderdeel van dit fellowship was een stage van één maand in Hospital for Sick Kids in Toronto, Canada, waar Prof. Anne Griffiths haar de fijne kneepjes van de klinische behandeling van pediatrische IBD bijbracht. Bij de aanvang van dit fellowship startte zij haar promotieonderzoek, eveneens in het Emma Kinderziekenhuis/AMC. In juli 2006 werd zij aangesteld als kindergastro-enteroloog in het VU Medisch Centrum te Amsterdam.

Lissy is getrouwd met Marcel Brouwer en heeft 2 dochters, Lara en Amber.

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