Surgery and immuno modulation in Crohn's disease

Pär Myrelid



Division of Surgery
Department of Clinical and Experimental Medicine
Faculty of Health Sciences
Linköping University
581 85 Linköping
Sweden

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On mice and men
Cover page: Intra-operative endoscopy during surgery for Crohn's disease of the small bowel
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"I don't mean to deny that the evidence is in some way very strong in favour of your theory. I only wish to point out there are other theories possible."

Sherlock Holmes

Adventure of the Norwood Builder Sir Arthur Conan Doyle

ABSTRACT

Crohn's disease is a chronic inflammatory bowel disease with unknown origin. This study investigates the combined use of surgery and immuno modulation in Crohn's disease. The outcome of medication and surgery in 371 operations on 237 patients between 1989 and 2006 were evaluated. Moreover the effects of prednisolone, azathioprine and infliximab on the healing of colo-colonic anastomosis in 84 mice with or without colitis were evaluated.

The use of thiopurines after abdominal surgery in selected cases of severe Crohn's disease was found to prolong the time to clinical relapse of the disease from 24 to 53 months. Patients on postoperative maintenance therapy with azathioprine had a decreased symptomatic load over time and needed fewer steroid courses.

The use of thiopurines was found to be a risk factor of anastomotic complications in abdominal surgery for Crohn's disease together with pre-operative intra-abdominal sepsis and colo-colonic anastomosis. The risk for anastomotic complications increased from 4 % in those without any of these risk factors to 13 % in those with any one and 24 % if two or three risk factors were present.

In patients with two or more of these, or previously established, risk factors prior to surgery one should consider refraining from anastomosis or doing a proximal diverting stoma. Another possibility is to use a split stoma in which both ends of a future delayed anastomosis are brought out in the same ostomy hole of the abdominal wall. This method was found to significantly decrease the number of risk factors prior to the actual anastomosis as well as decreasing the risk of anastomotic complications, without increasing the number of operations or the time spent in hospital.

In the animal model all three medications had an ameliorating effect on the colitis compared with placebo. Only prednisolone was found to interfere with the healing of the colo-colonic anastomoses with significantly decreased bursting pressure compared with placebo as well as azathioprine and infliximab.

The association between azathioprine therapy and anastomotic complications may be due to a subgroup of patients with a more severe form of the disease who have an increased risk of such complications and also are more prone to receive intense pharmacological therapy.

Vis är den som har som rättesnöre att tänka efter före

Tage Danielsson

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to by their Roman numerals as follows:

I. Azathioprine as a postoperative prophylaxis reduces symptoms in aggressive Crohn's disease

Pär Myrelid, Susanne Svärm, Peter Andersson, Sven Almer, Göran Bodemar, Gunnar Olaison.

Scand J Gastroenterol 2006;41:1190-1195.

II. Thiopurine therapy is associated with postoperative intraabdominal septic complications in abdominal surgery for Crohn's disease

Pär Myrelid, Gunnar Olaison, Rune Sjödahl, Per-Olof Nyström, Sven Almer, Peter Andersson Dis Colon Rectum 2009;52:1387-1394

III. Split stoma in resectional surgery of high risk patients with ileocolonic Crohn's disease

Pär Myrelid, Johan D Söderholm, Rune Sjödahl, Peter Andersson Submitted 2009

IV. Effects of anti-inflammatory therapy on bursting pressure of colonic anastomosis in dextran sulfate sodium colitis in mice Pär Myrelid, Sa'ad Salim, Silvia Melgar, Mihaela Pruteanu, Peter Andersson, Johan D Söderholm In manuscript 2009

ABBREVIATIONS

AIEC Adherent-Invasive Escherichia coli

AZA Azathioprine

BMI Body Mass Index

BP Bursting pressure

CD Crohn's Disease

CDAI Crohn's Disease Activity Index

CS Corticosteroids

DNBS Dinitrobenzene Sulfonic Acid

DSS Dextran Sulfate Sodium

ECCO European Crohn's and Colitis Organisation

FAP Familial Adenomatous Polyposis

HE Hematoxylin-Eosin

IASC Intra Abdominal Septic Complications

IFX Infliximab

IBD Inflammatory Bowel Disease

IL Interleukin

IPAA Ileal Pouch-Anal Anastomosis

MT Masson's Trichrome

MMF Mycophenolate Mofetil

MMP Matrix Metalloproteinase

6-MP 6-Mercaptopurine

MTX Methotrexate

OR Odds Ratio

PAS Periodic Acid-Schiff

SEMS Self Expanding Metal Stents

TIMP Tissue Inhibitor of Metalloproteinase

6-TG 6-Thioguanine

TNBS Trinitrobenzensulfonic Acid

TNFα Tissue Necrosis Factor α

TPMT Thiopurine S-Methyltransferase

UC Ulcerative Colitis

VAS Visual Analogue Scale

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Study in blue and green Jakob, 2

INTRODUCTION

Background

Inflammatory bowel disease is a disorder involving chronic intestinal inflammation and is composed of three major phenotypes, Crohn's disease, ulcerative colitis, and microscopic colitis. Crohn's disease is characterized by discontinuous transmural inflammation involving any portion of the gastrointestinal tract, with the ileum and colon most commonly affected^{6, 7}. In ulcerative colitis the inflammation is limited to the mucosa, and involving the rectum and, to a variable extent, the colon in a continuous manner⁶. In approximately 10 % of individuals, confirmed inflammatory bowel disease limited to the colon cannot be clearly classified as ulcerative colitis or Crohn's disease and then is labelled as indeterminate colitis¹³, this entity should however not be used until colectomy is performed and pathologists still are unable to define the diagnosis¹⁴.

Crohn's disease still has an unknown etiology and has its name after the first author of the paper "Regional ileitis: A pathologic and clinic entity" published in JAMA 1932¹⁵. However, two circumstances out of the ordinary gave the disease its eponym; first JAMA had a policy to list authors alphabetically rather than after the importance of their contribution and second the senior surgeon, Dr Berg, who was involved in the cases, was reluctant to have his name on a paper he hadn't written himself.

Burril B Crohn, Leon Ginzburg and Gordon D Oppenheimer were physicians active at Mount Sinai Medical Center in New York, in the 21st century still a center in the forefront of research in Crohn's disease. In the 1932 paper on the disease they described it as a regional chronic granulomatous inflammation of the terminal ileum leading to fibrosis and eventually even obstruction. This was not the first report of the disease but the previous reports were brief reports of limited number of patients 16-18. Actually the Scottish surgeon T Kennedy Dalziel was in some parts closer than Crohn and colleagues to today's concept of the disease in his description from 1913 as he was describing ileal and colonic lesions and compared it with Johne's disease in cattle, caused by *Mycobacterium paratuberculosis* which still is discussed as a possible etiologic factor 19.

Symptoms and Disease Manifestations

It was not until 1959 that the disease was shown not to be limited only to the distal ileum but appearing in the colon and anus as well²⁰⁻²².

Later it has been characterized as a pan enteric disease, ranging from the mouth to the anus (Figure 1), and associated with extra intestinal manifestations like arthralgia as well as eve-, skin- and livermanifestations^{23, 24}. The intestinal symptoms are dominated by abdominal pain and obturations but diarrhea and mucous in the stool, as well as fistulas, are quite frequent²⁴⁻²⁶. Many people with Crohn's disease have symptoms for years prior to the diagnosis²⁷ and diagnostics is often difficult since there is no gold standard²⁸. There is ongoing research to improve the diagnostics by invasive as well as noninvasive methods. Recently the development of a multi-gene expression algorithm analysis on biopsies from colonic mucosa showed promising abilities in discriminating between non-inflammatory bowel diseases as well as between ulcerative colitis and colitis in Crohn's disease²⁹. The final diagnosis will remain a test of clinical skill depending on relevant history, attentive physical examination, judicious laboratory testing, and detailed review of radiographic, endoscopic, and pathologic data²⁸.

The disease was first treated with extensive resection³⁰, with an inherent risk of developing short bowel syndrome and intestinal failure^{31, 32}. During the 1980's it was shown that residual microscopic disease at the margins of resection was of no influence on the time span until repeat surgery^{33, 34}. The extent of surgery has repeatedly been shown to be of less importance for treatment outcome³⁵⁻³⁷, and today inflamed areas are often not surgically removed and short strictures treated by stricturoplasty instead of resection³⁸⁻⁴¹.

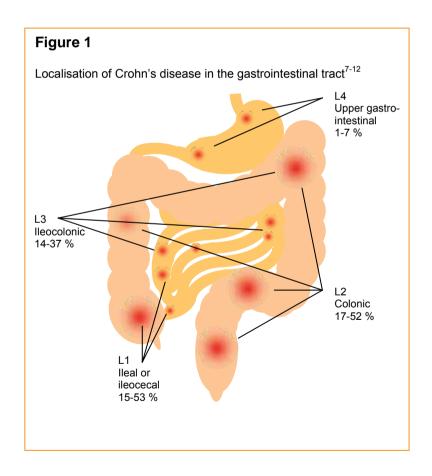
Inflammatory bowel disease is today classified according to the Montreal classification, which was revised 2005 and presented at the World Congress of Gastroenterology in Montreal¹⁴ (Figure 1 and Table 1). This classification tries to address the problems of patient counselling, assessment of disease severity and prognosis as well as guidance in finding the most appropriate therapy according to subtype. The classification has several limitations, the most prominent being disease behaviour since this is dynamic and seems to change over time⁴².

Table 1						
Montreal classification for Crohn's disease						
Age at diagnosis	A1 A2 A3	< 16 years 16-40 years > 40 years				
Location	L1 L2 L3 L4	Ileal Colonic Ileocolonic Isolated upper disease*				
Behaviour	B1 B2 B3 p	Non stricturing, non penetrating Stricturing Penetrating Perianal disease modifier:				
* L4 is a modifier that can be added to L1-3 when concomitant upper gastrointestinal disease is present. ± p is added to B1-3 when concomitant perianal disease is present.						

Extra intestinal manifestations occur in up to one third of patients with inflammatory bowel disease and patients with perianal Crohn's disease have an increased risk for such complications^{23, 43}. Arthropathy, cutaneous manifestations (erythema nodosum and pyoderma gangrenosum), and eye manifestations (episcleritis and uveitis) being the most common^{43, 44}. Other diseases are also found more frequently than expected, like hepatobiliary disease (primary sclerosing cholangitis), osteporosis and atopic disorders (eczema)^{23, 43-46}.

There has been a concern regarding an association of inflammatory bowel disease and the development of cancer. Inflammatory bowel diseases have a marginally increased risk of haematopoietic cancer and Crohn's disease constitutes a modest increase in the risk for lymphoma^{47, 48}. A risk of developing colorectal cancer has been shown in patient with ulcerative colitis, especially those with early onset and long disease duration, pancolitis, primary sclerosing cholangitis and/or first degree relatives with sporadic colorectal cancer ⁴⁹⁻⁵¹. The risk of colorectal cancer in Crohn's disease is still debated ⁵²⁻⁵⁷, but meta analyses have found 1.9-2.9 times increased risk of developing colorectal cancer in Crohn's patients ⁵⁷⁻⁵⁹. This is also similar to a recent study from the United Kingdom by Goldacre *et al* ⁶⁰ and recently surveillance has been recommended by the European Crohn's and

Colitis Organisation (ECCO)⁶¹. There seems to be a 10-60 fold increased risk of small bowel carcinoma, however still very infrequent since it is an uncommon type of cancer to start with^{51, 55, 57, 62, 63}, and a significant association between the disease in a certain segment of the bowel and the risk of developing cancer in the same segment is evident⁵⁹. There is an emerging interest in potential chemo-preventative strategies in both sporadic and colitis-associated colorectal cancer and there have been suggestive data that chronic maintenance therapy with 5-aminosalicylates ^{50, 55} might reduce the risk of developing colorectal cancer as well as small bowel cancer⁶⁴. If this is an effect of the medication itself or merely an effect of decreased inflammatory activity is unclear, as is the potential effect of thiopurines and newer anti-inflammatory drugs⁶⁵.



Mortality

Crohn's disease is a chronic disease that strikes early in life entailing risks for severe complications from the disease itself as well as from the medical and surgical therapies⁶⁶. In a study from Stockholm an increased mortality risk was seen, with 93.7 % of the expected survival after 15 years follow up⁶⁷. The only factor in this study separating Crohn patients from the background was death from gastrointestinal disorders, others than inflammatory bowel disease. A close to doubled incidence of suicide was also seen, which also have been found in prior reports⁶⁸, but did not reach significance. Other studies have not found an overall increased mortality risk except for an increment during the first five years after diagnosis in patients during their twenties as well as in patients with extensive small bowel disease⁶⁹.

Quality of life

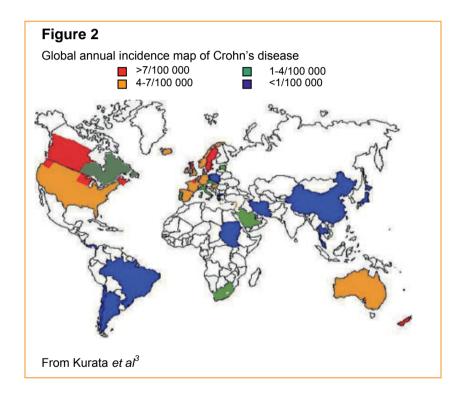
Being a chronic disease it may have a severe impact on quality of life compared with the general population, including concerns regarding a possible need for an ostomy, the uncertain nature of disease, and lack of energy⁷⁰⁻⁷². However, studies have shown that the impairment in quality of life is associated with the disease activity rather than the disease itself and its localisation or behaviour^{71, 73-78}. By using medical therapy and surgery as complementary treatments you can reach a high number of patients in remission with low symptomatic load^{79, 80}. To further emphasize the correlation between remission and quality of life Casellas *et al* the found no difference in quality of life between medically or surgically induced remission⁸¹ and in patients with severe perianal Crohn's disease Kasparek *et al* found a better quality of life in those receiving a diverting stoma⁸². Other studies have not focused on severity of symptoms but rather the doctor-patient interaction, and it seems that a lot could be gained in the area of supportive care⁸³.

Epidemiology

Crohn's disease is a disease of the industrialised world with increasing prevalence with a south-north and east-west gradient and with the highest prevalence in Scandinavia, the United Kingdom and North America (Figure 2). In North America there is also a difference among different ethnic groups with a prevalence of only 4-5/100 000 among Asians and Hispanics compared to 29.8 and 43.6 for African-Americans

and Caucasians respectively³. Individuals with Ashkenazi Jew ancestry is also a group with a two- to nine-fold increased risk of inflammatory bowel disease⁸⁴.

Crohn's disease has a bimodal distribution in incidence as a function of age^{3, 12}. The disease tends to strike people in their teens and 20s, and people in their 50s¹². It is rarely diagnosed in early childhood but recent studies show an increase in all ages^{11, 85}. Among children a shift from ulcerative colitis towards Crohn's disease is seen as well as a net increase in inflammatory bowel disease as a group^{85, 86}.



Etiology

The etiology of Crohn's disease is still not clear. It is today looked upon as not caused by a single factor, but rather by a combination of genetic susceptibility, environmental, immuno-regulatory, and epithelial barrier factors⁸⁷.

Food and lifestyle

The rising incidence of inflammatory bowel disease coincides with a definite change of the dietary pattern, especially in the westernized world⁸⁸ and hence food (e.g. refined sugars)⁸⁸⁻⁹⁰, fast food⁹¹ smoking^{90, 92-} ⁹⁴ and birth control pills⁹⁴ among others have been suggested to have a role in the aetiology of Crohn's disease. It is hard to tell if for instance an increased intake of sugar among Crohn's patients is secondary to the disorder or in fact a partial cause of the disorder^{88, 95}. Reif et al^{96} tried to evaluate the pre-diagnosis consumption in newly diagnosed patients and found increased intake of sugar, however inevitably retrospective data. Smoking as a risk factor has even shown a dose response relationship⁹⁰ as well as a capability to influence the severity of the disease and the risk of surgery^{97, 98}. In a study from 2001 by Cosnes et al ⁹⁹ smokers were compared with non-smokers and those who recently guit smoking (and maintained that status for more than a year). No differences were found between ex-smokers and non-smokers, but both these groups differed from the smokers in regards of flare ups and need for medical therapy during a 2.5 year period of follow up.

Genes

Studies on monozygotic (identical) and dizygotic (non-identical) twins and their concordance were the first attempts to really evaluate the concept of genes and inheritance of inflammatory bowel disease. If the disease was to be entirely genetic the concordance in monozygotic twins would approach 100 % and that in dizygotic twins 50 %. In large studies from Scandinavia and the United Kingdom the concordance rate for Crohn's disease in monozygotic twins was 20-50%, whereas the concordance rate in dizygotic twins brought up in the same environment was less than 10 % 100-103. Also within families there has been a risk increment for inflammatory bowel disease. Patients with Crohn's disease report a history of inflammatory bowel disease in a first degree relative in 5.2-15.6 % for any kind of inflammatory bowel disease and 2.2-13.6 % for Crohn's disease 104.

The NOD2/CARD15 gene locus was in 2001 the first identified locus in individuals predisposed to Crohn's disease^{105, 106} and today more than 30 independent loci have been identified as being associated with Crohn's disease¹⁰⁷. CARD15 variants are found in the majority of Caucasian CD patients and vary between 35-45 % with the exception of Scandinavian, Irish and Scottish patients, where the prevalence is much lower¹⁰⁸. The mutation frequency for NOD2/CARD15 was found to be high both among twins with Crohn's disease and their healthy siblings¹⁰⁹.

Since the discovery of NOD2/CARD15 genome scans have generated more than ten regions which have led to the identification of a number of susceptibility genes besides CARD15 (DLG5, OCTN1 and 2, NOD1, HLA, TLR4, MAGI2)^{108, 110}. The NOD2 codes for a protein involved in recognition of bacteria in monocytes, macrophages, dendritic cells, epithelial cells and Paneth cells. A lot less is still known about the other susceptibility genes involved in Crohn's disease. HLA genes encode cell surface glycoproteins which, as in the case of NOD2 protein, are expressed on antigen presenting cells, and are involved in the T cell activation through presentation of peptides to T cell receptors¹¹¹. DLG5 and MAGI2 encodes scaffolding proteins involved in epithelial integrity, thus supporting the significance of the epithelial barrier in IBD pathogenesis¹¹⁰.

Mucus

Mucins are the primary constituents of extra cellular mucus at the cellular barrier. They are usually very large, filamentous molecules with molecular weights up to several million Daltons and are important epithelial products of the intestine and essential for a functioning epithelial barrier¹¹². Moreover, mucins are very important in the contact of many micro-organisms with the intestinal mucosa and a primary defect in mucins could breach the epithelial barrier through altered mucosal–bacterial interactions.

Smoking generally increases mucus production within the body and a decrease is seen in ulcerative colitis compared to controls and similarly an increased risk of developing ulcerative colitis is a well known phenomena after smoking cessation¹¹³. In Crohn's disease on the other hand the mucus layer is found to be thicker than normal^{114, 115} and an inverse effect of smoking is seen as well^{90, 92, 93}.

Reduced expression of MUC1, MUC3, MUC4, and MUC5B has been shown in Crohn's disease and the membrane bound MUC3 and the secretory MUC2, are clearly involved in the pathogenesis of inflammatory bowel disease¹¹². A mouse model with MUC2 mucin knockout mice showed histological loss of the characteristic colonic goblet cells shape in the absence of MUC2 as well as being more susceptible to dextran sulfate sodium-induced colitis¹¹⁶. A high detectability of MUC2 protein in both UC and CD seems not due to increased transcription of MUC2 mRNA, but is rather caused by an altered post-transcriptional process, involving diminished sulphation and/or glycosylation of the protein¹¹⁷. A significant down regulation in the colon in Crohn's patients was obtained for MUC2 and MUC12 and these

alterations, leading to shortening of the carbohydrate chains, may prevent effective gel formation¹¹⁸.

Mice with a missense mutation in the MUC2-gene showed aberrant MUC2 biosynthesis, less stored mucin in goblet cells, a diminished mucus barrier, and increased susceptibility to DSS-induced colitis. Enhanced local production of IL-1 β , TNF- α , and IFN- γ was seen in the distal colon, and intestinal permeability increased twofold. The number of leukocytes within mesenteric lymph nodes increased fivefold and leukocytes cultured in vitro produced more Th1 and Th2 cytokines (IFN- γ , TNF- α , and IL-13)¹¹⁹. In patients with ileal Crohn's disease the expression of MUC1 mRNA was found to be decreased when compared to healthy mucosa. The expression levels of MUC3, MUC4, and MUC5 were also significantly lower in inflamed as well as normal mucosa in patients with Crohn's disease compared with healthy controls suggesting a mucosal defect on a genetic basis in Crohn's disease¹²⁰.

Stress

Crohn's disease has by some authors been suggested to be a psychosomatic illness. Other reports found a difference in personality profile with high anxiety score correlating to the duration of the disease rather than disease itself but there are also contradictory findings with no differences in patients with Crohn's disease compared to the general population¹²¹⁻¹²⁴. Nevertheless approximately 50-75 % of patients with Crohn's disease believe a stressful life event or a nervous personality to be involved in triggering a relapse of the disease^{123, 125}.

Animal models of colitis are fairly consistent in identifying increased epithelial permeability secondary to stress as a factor mediating the relationship between stress and inflammation. Qiu *et al* described a paradigm of experimentally dinitrobenzene sulfonic acid (DNBS) induced colonic inflammation in mice. After resolution of the acute inflammation, colitis was reactivated by the combination of a sub-threshold dose of DNBS and stress but neither sub-threshold DNBS alone nor stress alone would reactivate the colitis. Stress reduced colonic mucin and increased colonic permeability¹²⁶.

Metabolically stressed epithelium displays increased permeability in the presence of viable non-pathogenic *Escherichia coli* and is further exaggerated by TNF- α release by activated immune cells¹²⁷. Such stress makes the mucosa perceive normally harmless bacteria as threatening, resulting in loss of barrier function, increased permeability of bacteria, and increased chemokine synthesis¹²⁸. In rats luminal horseradish

peroxidase was absorbed more readily into the mucosa of stressed animals, regardless of acute or chronic stress ^{129, 130}. The stressed animals also showed an increased expression of IL-4 and a decreased expression of IFN-γ in the mucosa while treatment with a corticotropin-releasing hormone antagonist eliminated these manifestations, indicating that the presence of an oral antigen during chronic psychological stress may alter the immune response ¹³⁰. Chronic stress also induced an over thirty fold increase in the transit of *Escherichia coli* across the follicle associated epithelium ¹²⁹. The barrier function of follicle associated epithelium can accordingly be modulated by chronic stress, enhancing the uptake of luminal antigens and bacteria, which may have implications in the initiation of the pro-inflammatory immune response within the intestinal mucosa ^{129, 131-133}.

Permeability

Increased intestinal permeability may be stress induced in healthy controls as well as in inflammatory bowel disease 126-128, 130, 133, 134 but is also seen in other chronic inflammatory disorders, like asthma and coeliac disease 135, 136. In a study of patients with Crohn's disease, their relatives, spouses as well as healthy controls the permeability of the mucosa was determined at baseline as well as after provocation by acetylsalicylic acid. Patients had significantly higher permeability compared with the controls and their relatives. After provocation by acetylsalicylic acid the permeability increased in all groups, but significantly more among patients and their relatives compared with the other two groups. This suggest that baseline permeability is determined by environmental factors while permeability after provocation is a function of a genetically determined state of the mucosal barrier¹³⁷. Noninflamed ileal mucosa from patients with Crohn's disease did not differ from controls with colonic cancer while inflamed specimens showed a significantly increased permeability¹³⁸. After luminal antigen exposure the permeability increased in non-inflamed areas also suggesting an important connection between luminal stimuli and the epithelium in the pathogenesis of Crohn's disease. The increased endosomal uptake of antigens in histologically non-inflamed ileum of patients with Crohn's disease has also been shown to be regulated by TNF- α^{139} . Patients with long standing Crohn's disease differ from ulcerative colitis in regards of transmucosal bacterial uptake across the follicle associated epithelium followed by an increased co-localization between such bacteria and dendritic cells and an increased release of TNF-α which might initiate and/or perpetuate an inflammation of the gut^{140, 141}. Other suggested mechanisms involved in the increased permeability of the intestine are abnormal tight junctions, bacterial α-hemolysin induced focal leaks,

apoptotic leaks in the mucosa, transcytotic antigen uptake mechanisms, and mucosal gross lesions¹⁴²⁻¹⁴⁴.

Micro-organisms

Different agents have been proposed to be responsible or associated with the development of Crohn's disease through an improper host response to normal enteric bacteria¹⁴⁵. Partly due to the clinical and histological resemblance with Crohn's disease the most likely bacteria for long has been the *Mycobacterium avium* subspecies paratuberculosis¹⁴⁶. Even *Helicobacter pylori* has been a causative candidate¹⁴⁷. *H. pylori* has been shown too cause gastritis and peptic ulceration in the stomach and duodenum¹⁴⁸, at first questioned but later rendering a Nobel prize in 2005. Keeping that story in mind it might be too early to rule out the role of bacteria in Crohn's disease yet.

The last few years numerous reports have shown increased numbers of mucosa-associated adherent-invasive *Escherichia coli* (AIEC) in patients with inflammatory bowel disease which further has been shown capable of infecting macrophages and leading to an increased secretion of TNF- $\alpha^{132, 139}$. Another finding focusing on the importance of microbia in the ethiology of inflammatory bowel disease is the anti-inflammatory effect of *Faecalibacterium* prausnitzii¹⁴⁹. A reduced proportion of *F. prausnitzii* in the normal ileal flora has been shown to increase the risk of endoscopic recurrence six months after surgical resection for Crohn's disease. The anti-inflammatory effect of *F. prausnitzii* is further shown through the reduced severity of TNBS-induced colitis in mice after oral administration of the bacteria¹⁴⁹.

Ekbom *et al* found an association between increased incidence of Crohn's disease and previous outbreaks of measles¹⁵⁰. A number of studies using polymerase chain reaction technique for viral expression dignosis on biopsies of resected specimens with Crohn's disease have shown diverting findings with an overweight towards a negative association¹⁵¹.

Appendicitis

Appendectomy for an inflammatory condition before the age of 20 is associated with a low risk of subsequent ulcerative colitis^{152, 153}. On the other hand, among patients with a history of appendectomy an increased risk of Crohn's disease is found which is continuously present up to 20 years after the appendectomy with an incidence rate ratio (95 % CI) of 1.47 (1.24–1.73) for any appendectomy and 2.11 (1.21–3.79) for

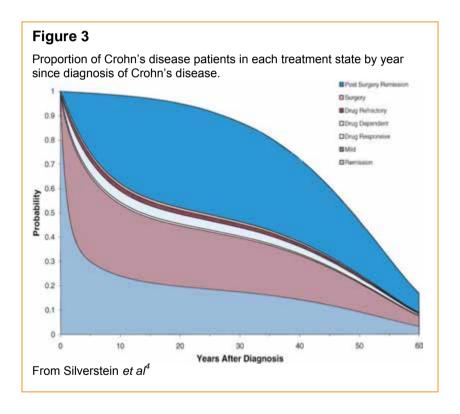
perforated appendicitis¹⁵⁴. The same study also found a worse outcome in Crohn's disease patients operated for a perforated appendicitis with an increased incidence rate ratio of intestinal resections of 2.7 (1.9–4.0). However, these findings are controversial and a possible common etiology unknown. In a more recent study from Sweden and Denmark only a transient increased risk was found during the first 5-10 years after appendectomy, with the exception of appendectomy in patients without appendicitis or mesenteric lymphadenitis, altogether suggesting that this seemingly increased risk might only be a diagnostic bias of incipient Crohn's disease¹⁵⁵.



Family and girl with guts
Hanna, 6

BACKGROUND TO THE STUDY

Since there presently is no cure for Crohn's disease all therapies are focused on relief of symptoms and dealing with complications to the disease. In active disease, induction of remission is achieved either through medical therapy or surgery. When patients have no or limited symptoms therapies are aimed at maintenance of remission, to prevent relapse of symptoms. It is important to remember that most of the clinical course is spent in remission as described in figure 3⁴.



Medical Treatment

Steroids

Corticosteroids including newer compounds like budesonide (Entocort®), with less systemic side effects¹56, ¹57, have a very good effect in induction of remission¹56, ¹58-¹60 but no effect in maitaining remission over a longer period of time¹56, ¹59, ¹6¹. Still some patients are not able to wean off their

steroid treatment and become steroid dependent ¹⁶². A major problem with steroids over a longer period of time are side effects, like osteoporosis ¹⁵⁶.

Aminosalicylates

Sulfasalazine, developed by Nanna Swartz – founder and first chairman of the Swedish Society of Gastroenterology¹⁶³, and the newer types of 5-aminosalicylates like mesalazine and olsalazine have a proven effect in ulcerative colitis, as induction of remission¹⁶⁴ as well as maintenance therapy¹⁶⁵. This positive effect has however not been shown in Crohn's disease^{166, 167}. They are, despite the lack of evidence, still often used¹⁶⁸.

Antibiotics

A numerous amount of studies on different antibiotics have been performed during the years 145, 146. Most studies are small, retrospective, short term or with a high number of drop outs. The majority of studies showing a positive effect seem to do so mainly in colonic Crohn's disease 145, 146. The antibiotics metronidazole and ciprofloxacin have been shown to have effect on fistulising perianal Crohn's disease but no sustained effect in luminal disease 169-171. Continuous therapy with ornidazole during one year after surgery have been shown to have a positive effect in preventing recurrences but this effect diminished after cessation of the therapy 172. In a recent report from the same group there seems to be a promising use of a combination of metronidazole (first three months) and azathioprine (twelve months) in regards to less endoscopic recurrences during the first year after ileocecal resection 173.

Immuno modulators

Thiopurines are anti-metabolites developed during the 1950's by Gertrude Elion, who later was awarded the Nobel Prize. It consists of three different drugs, 6-mercaptopurine (Puri-nethol®), azathioprine (Imurel®), and 6-thioguanine (Lanvis®). They were first used as chemotherapy in cancer and today widely used as immuno modulators in transplantation as well as in inflammatory bowel disease¹⁷⁴⁻¹⁷⁸.

Azathioprine has been endoscopically shown to heal the mucosa of both ileitis and colitis in Crohn's disease^{179, 180} and the place of thiopurines as maintenance therapy in inflammatory bowel disease is well established^{176, 177} and increasingly used over the years^{181, 182}. Healing of mucosal lesions one year after initiation of medical therapy has been found to predict a favorable five year outcome in terms of decreased inflammation, need for repeat steroids and resectional surgery¹⁸³. A

Cochrane analysis showed a number needed to treat of six on quiescent disease but also a steroid sparing effect with a number needed to treat of three¹⁷⁸. A study by Markowitz *et al* on children with newly diagnosed Crohn's disease speaks in favor of starting thiopurines earlier in the course of the disease¹⁸⁴. To verify this finding several studies are ongoing in adults as well¹⁸⁵. A recent French study found a high relapsing risk of Crohn's disease if the thiopurine therapy was interrupted, thus suggesting the thiopurine therapy not to be withdrawn if once tolerated¹⁸⁶.

However, approximately 15 % of patients have to end their thiopurine therapy because of side effects¹⁸⁷. Both 6-mercaptopurine and azathioprine are pro-drugs that are activated in the body through extensive metabolism¹⁸⁸. Measurements of thioguanine nucleotides have been tried for finding the right dosage and monitoring the use of thiopurines but with limited value¹⁸⁷. There is however data in favor of phenotyping and/or genotyping the catabolic enzyme thiopurine S-methyltransferase (currently 23 genetic variants have been described) prior to thiopurine therapy is commenced in inflammatory bowel disease to prevent severe haematotoxicity¹⁸⁹⁻¹⁹¹. All intolerance is not dosedependant and it seems that some patients not tolerating azathioprine do tolerate a switch to 6-mercaptopurine, and in some extent 6-thioganine^{187, 192}.

Mycophenolate mofetil, tacrolimus, cyclosporine A, and methotrexate are other immunomodulators used with variable success in inflammatory bowel disease ¹⁹³⁻²⁰³ and are so far regarded as a third line therapy in patients intolerant to thiopurines ²⁰⁴⁻²⁰⁶.

Surgeons have been concerned that immuno modulation with e.g. thiopurines will increase the risk of anastomotic complications²⁰⁷, through mechanisms of impaired healing. This potential impairment of the healing capacity by immuno modulation may be related to decreased proliferation and increased apoptosis of epithelial cells; it may also be related to T-cell-mediated suppression of the inflammatory reaction, which would lead to the impairment of collagen synthesis and wound strength^{208, 209}. The knowledge in this respect is however very limited. The reports are often focused on postoperative septic complications in general and do not always distinguish between Crohn's disease and ulcerative colitis patients²¹⁰⁻²¹².

Biologics

The first reports on biological therapy was in 1995 by van Dullemen *et al* using infliximab (Remicade[®]), a chimeric anti-TNF antibody of mouse origin that has been humanized²¹³. Later several reports as well as meta-analyses have shown their efficacy in induction^{214, 215} as well as maintenance of remission²¹⁶. Like in the case of thiopurine therapy clinical improvement after infliximab is accompanied by significant healing of endoscopic lesions and diminished mucosal inflammatory infiltration²¹⁷ and scheduled maintenance therapy seems to be more efficacious than episodic symptom driven therapy^{218, 219}. Frøslie *et al* showed the value of mucosal healing as a prognostic marker in long-term Crohn's disease¹⁸³. Even though this study was completed before the era of biologics the mucosal healing seems to be an important factor in evaluating therapies and has recently been shown valid in biological therapy as well^{183, 219}.

There have been reports on severe adverse events during biological treatment with e.g. infections, congestive heart failure, intestinal obstruction and lymphomas as well as infusion reactions²²⁰⁻²²². Today a latent tuberculosis infection must be ruled out before commencing anti-TNF-therapy²²¹ and in rheumatoid arthritis concomitant low dose steroids tend to ameliorate infusion reactions and decrease the risk of withdrawal²²³. The use of per oral steroids and/or anti-histamines is also applied quite frequently during anti-TNF therapy in Crohn's disease²²⁴.

A few years back a completely human antibody, adalimumab (Humira®), also received approval for therapy in Crohn's disease²¹⁶. This drug can be given as a sub cutaneous injection rather than intravenously, like infliximab, and carries a lower risk of infusion reactions²²⁵. Both drugs are quite costly but a recent statistical simulation speaks in favour of their cost-effectiveness for up to four years of continuous therapy considering a lifetime perspective²²⁶.

An ongoing discussion is whether biological therapy should be combined with immuno modulation or not as a standard therapy. A recent study on subgroups from four large studies on infliximab, two on ulcerative colitis and Crohn's disease (where close to 40 % of the patients on biological therapy received concomitant immuno modulation) did not improve efficacy or pharmacokinetics²²⁷. In another recent study patients on combined therapy with infliximab and thiopurines were randomised to continued therapy or single therapy with infliximab which showed no obvious differences between the two regimens²²⁴. However, an improvement in the rate of infusion reactions was seen in the group receiving the combined therapies^{227, 228}.

Natalizumab (Tysabri®), a recombinant humanized IgG4 monoclonal antibody that inhibits the migration of mononuclear leukocytes into areas of inflamed tissue, is approved for multiple sclerosis. However, it has been shown to have an effect in induction of remission of Crohns disease but with an increased risk for severe adverse events, e.g. progressive multifocal leukoencephalopathy (PML) a potentially lethal condition²²⁹ and is therefore not approved for inflammatory bowel disease in Europe.

Ustekinumab (Stelara[®]), a monoclonal antibody against the common p40 unit of interleukin-12 and -23, has been used with efficacy in psoriasis²³⁰ and recently also shown to induce a clinical response in moderate-to-severe Crohn's disease²³¹.

Other medical therapies

Other therapies have been tried as well, such as omega 3 fatty acids (e.g. fish oil)^{232, 233} and helminth therapy (e.g. hookworm) with *Necator americanus* larvae or *Trichuris suis* eggs²³⁴, but have not found a place in the common therapeutic arsenal.

Non Pharmacological Treatment

Apheresis is a quite new therapeutic modality where the mechanism is to a large extent unknown. There are two different versions on the market so far, Addacolumn[®] and Cellsorba[®]. Addacolumn[®] is a column of cellulose acetate beads and Cellsorba[®] is a filter, in both cases two of the postulated mechanisms are extra corporal depletion of activated immune cells and modulation of the cytokine response. Both systems have a more wide spread use in ulcerative colitis but their true efficacies are still unknown²³⁵⁻²³⁷.



Dad

Ella, 9

Surgical Treatment

Despite the development of new pharmacological therapies there is still a need for surgery 181. Crohn's disease has typical features during surgery; serositis, fatty wrapping and thickening of the intestinal wall²³⁸. During the 1950's when U.S. president Dwight D. Eisenhower was operated on for Crohn's disease, resections were associated with a high risk of complications and bypass of the diseased segment was a common procedure²³⁹. Surgery in Crohn's disease is still known to be associated with a higher complication rate than surgery for other intestinal disorders²⁴⁰. This increased risk is partly caused by factors inherent to the disease itself, e.g. preoperative intra-abdominal sepsis, impaired nutritional status as well as medical therapy²⁴¹⁻²⁴⁴. Later the surgical technique and perioperative care was improved and resectional surgery became the standard procedure²⁴⁵⁻²⁴⁷. At that time "radical" resections, including wide margins of normal unaffected bowel on each side, became widely used in order to try to postpone post surgical recurrence^{246, 247} and some surgeons even made frozen sections intraoperatively to make sure resectional margins were free from inflammation³³. A few years later a repeated number of reports proved this wrong and that a conservative resection did not increase the risk of complications to the surgery or the risk of recurrence^{33, 35, 248}.

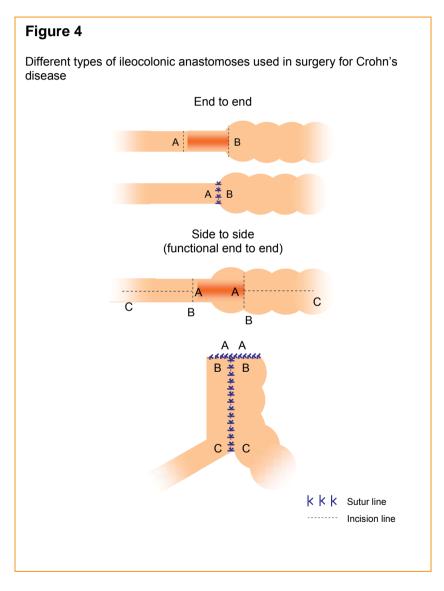
Three out of four patients with Crohn's disease will undergo an intestinal resection and half of them will ultimately relapse^{249, 250}. In the report from Bernell *et al* from Stockholm on Crohn's disease in general the cumulative rate of intestinal resection was 44 %, 61 %, and 71 % at 1, 5, and 10 years after diagnosis^{249, 250}. While surgery was the treatment of choice for *cure* of the disease by Crohn in his original work¹⁵ recurrence after surgery is a common feature of Crohn's disease^{249, 250}. Recurrences are often described as clinical (symptomatic), surgical (need for repeat surgery), or endoscopic (visible inflammation at endoscopy)²⁵¹.

Endoscopic recurrence may be demonstrated already within three months after an ileocolic resection, indicating Crohn's disease as a chronic intestinal process²⁵². There are also numerous reports on the value of endoscopic relapses as a predictor of symptomatic and/or surgical relapses, thus being a method of early detection of patients with an increased risk²⁵²⁻²⁵⁵. Recently the faecal biomarkers calprotectin and lactoferrin have been used to identify inflammatory disease recurrence in symptomatic postoperative patients as well²⁵⁶. In the previously mentioned work by Bernell *et al* postoperative recurrences occurred in 33 % and 44 % at 5 and 10 years after primary resection^{249, 250}. Similar figures were seen in the material from Gothenburg where the cumulative

risk for a repeat resection was 40 % after 10 years and 45 % after 15 years. The risk for having a third and fourth resection was 50 % of those having a repeat resection after 10 years²⁵⁰. In ileocecal Crohn's disease Bernell et al found resection rates 1, 5 and 10 years after diagnosis of 61 %, 77 %, and 83 % respectively while surgical relapse rates were 28 % and 36 % after 5 and 10 years, respectively, from the first resection²⁵⁷. In Crohn's colitis the gold standard, and still advocated by some²⁵⁸, used to be subtotal colectomy or procto-colectomy²⁵⁹, but recently segmental resections has been performed without increased risk together with better bowel function 260, 261. In patients with two or more colonic segments involved time to recurrence was postponed by more than four years in the group receiving ileorectal anastomoses compared with segmental resections. However, no significant differences were seen in regards of need for repeat surgery or stoma²⁶². Even when colectomy is needed an ileo-rectal anastomosis should be considered in cases with relative rectal sparing and without severe perianal disease in order to avoid or at least postpone the need for stoma^{263, 264}. The ileal pouch-anal anastomosis (IPAA) has been considered contra-indicated in Crohn's disease for a long time. Some patients thought to have ulcerative colitis receiving IPAA have later been diagnosed having Crohn's disease. Today it is considered to be a possible choice even in Crohn's colitis but patients should be informed of a higher risk of failure as well as poorer functional outcome (e.g. urgency and incontinence) compared with IPAA performed because of e.g. ulcerative coloitis or familial adenomatous polyposis (FAP)²⁶⁵.

An increased risk of having a surgical recurrence has been postulated in patients with female gender, early onset of the disease, perforating disease, perianal fistulas and in patients with ileal or ileocolic disease. especially those having a long segment resected due to the disease²⁴⁹, ^{257, 266}. In three recent studies patients with postoperative complications also seemed to have an increased risk of early relapse of the disease²⁶⁶-²⁶⁸, probably due to the fact that postoperative complications are signaling a more aggressive disease. Smokers with Crohn's disease have been shown to have an increased risk of clinical as well as surgical relapse with a odds ratio of up to 2.56 at 10 years 97, 98. It has been shown that higher mucosal levels of TNF-α and an increased state of activation of mononuclear cells in the lamina propria in patients with inactive Crohn's disease are significantly associated with an earlier clinical relapse of the disease as well²⁶⁹. Different surgical methods have been tried to decrease the risk of surgical relapses. In the 1980's a different anastomosis in ileocolonic resections was tried here in Linköping: creating a nipple in order to mimic the normal anatomy and prevent the colo-ileal reflux²⁷⁰, a method recently rediscovered²⁷¹.

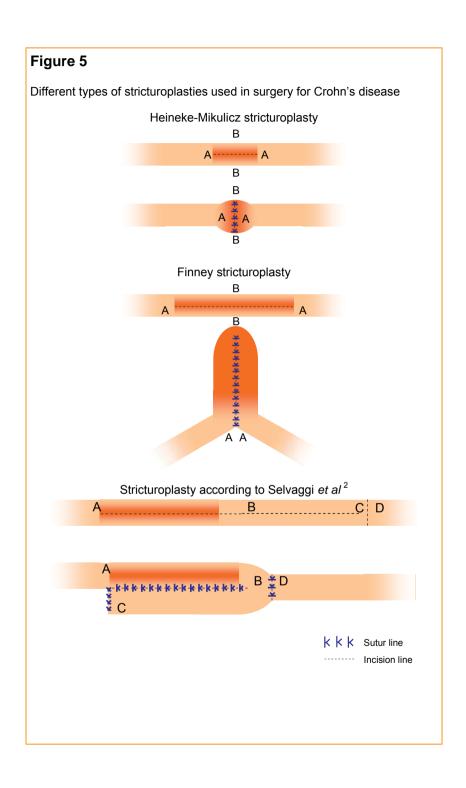
Different medical maintenance therapies, in order to maintain remission after surgery, have been evaluated as well. Most therapies seem to be of limited value (e.g. 5-ASA and steroids)^{156, 272, 273}, apart from the antibiotic ornidazole that has been shown effective during the first year after surgery for ileocolonic Crohn's disease¹⁷². The use of thiopurines after medically induced remission is well established¹⁷⁸ while the use as postoperative prophylaxis is less evaluated²⁷⁴.



Due to the fact that most stenotic recurrences develop at the site of the anastomosis 252, 253, 275 stapled side-to-side anastomoses were initiated in

order to make a wider anastomosis and thus postpone the stricturing. A number of retrospective reports have been showing mainly positive effects on the surgical recurrence rates after stapled side-to-side anastomoses²⁷⁶⁻²⁸¹ while a meta analysis showed a significantly lower anastomotic leak rate in the group receiving side-to- side anastomoses (OR 4.37: 95% CI, 1.3–14.7) but no significant difference in anastomotic recurrence or reoperation needed because of anastomotic recurrence²⁸². In a recent study by Scarpa et al a significantly lower incidence of recurrences, in regards to repeat surgery, was seen among patients with side-to-side anastomoses (regardless if stapled or hand-sewn) compared to a group of stapled end-to-side anastomoses^{266, 283}. thus speaking in favour of a wide lumen anastomosis rather than a stapled (Figure 4). In another study by the same authors they found a five year surgical recurrence rate of 30% in patients with an end-to-side anastomosis but only 6% in those receiving a side-to-side anastomosis²⁸⁴. On the other hand, a recent randomized multicenter study showed no differences in either endoscopic or symptomatic recurrences between a wide lumen stapled anastomosis or an end-toend hand-sewn anastomosis, the follow up period was however only one vear²⁸⁵.

In order to diminish the risk of developing short bowel syndrome in patients with widespread enteritis or multiple strictures³², stricturoplasties have been used with good results^{41, 286, 287}. In regards to quality of life no differences have been seen after stricturoplasty compared with resection²⁸⁸. The Heineke-Mikulicz stricturoplasty is usually used for short strictures (up to approximately 10 cm in length) and the Finney stricturoplasty is used for longer strictures (up to approximately 25 cm)⁴¹ but there has also been a development of more non-conventional methods² (Figure 5). Even though there are some reports on the development of cancer in stricturoplasties it seems safe²⁸⁹⁻²⁹¹, and a recent report from Yamamoto et al found cytokine production in biopsies from stricturoplasties to decrease to the same level as macroscopically normal ileal mucosa one year after stricturoplasty²⁹². Resection seems to protect against small bowel cancer⁶⁴, but in widespread disease the adverse effects of resecting large amounts of small bowel must be weighed against the relative small risk of developing small bowel cancer. In a similar manner colonic resection seems to be protective in colorectal cancer⁶⁰ but colonic segmental resections have less impairment of the bowel function^{260, 261}. No difference is seen in clinical outcome or quality of life between colonic resection or stricturoplasty²⁹³ why segmental resection, and not stricturoplasty, should be used in Crohn's colitis⁴¹.



During the last couple of years a number of reports have been showing advantages during the post-operative period in laparoscopic ileocolonic resections for Crohn's disease compared with open surgery²⁹⁴⁻²⁹⁶, even feasible in more complex Crohn's disease in experiences hands²⁹⁷. Regarding the long term outcome there is still not clear evidence wether laparoscopy has an advantage, other than cosmetic and with a decreased risk of incisional hernias, or not^{296, 298-301}. Quality of life has not been shown to be affected by the method of surgery, rather, it is the occurrence of a symptomatic recurrence in itself that impairs quality of life in patients previously in remission^{295, 302}.

As a result of a more conservative surgical strategy dilatation of strictures has been used as a complement to surgery³⁰³, and is a relatively safe procedure with high success rates in the case of short strictures (≤ 4 cm)^{304, 305}. Dilatations should however be performed with the possibility to take the patient to the operating theatre since there is a risk of perforation during dilatation ranging from 0-11 %^{304, 305} and the risk is higher in primary strictures compared to anastomotic strictures³⁰⁶. A handful of reports on the use of self expanding metal stents (SEMS), as a bridge to surgery as well as single therapy instead of surgery, have shown diverging results and even severe complications like perforation and fistula formation³⁰⁷⁻³⁰⁹. The use of SEMS in Crohn's disease needs to be further evaluated before it can be recommended but there may be a place for it in a palliative setting in patients with Crohn's disease unfit for surgery and with a limited life expectancy³¹⁰.

As with all kinds of surgery there is a risk of complications and in Crohn's disease this risk seems to be increased compared with colorectal surgery in general^{240, 311, 312}. Surgery in Crohn's disease is often performed on patients with a number of established risk factors such as the presence of preoperative malnutrition, intra-abdominal abscesses or fistulas, bowel obstruction, steroid treatment, and possibly immunomodulation^{210, 241, 243, 313, 314}. Surgeons have been concerned that such treatment will increase the risk of anastomotic complications through mechanisms of impaired healing²⁰⁷. Evidence, in this respect, is however limited. Previous reports are often focused on postoperative septic complications in general and do not always distinguish between surgery in Crohn's disease and in ulcerative colitis^{210, 315, 316}. Preoperative steroid treatment and its association with anastomotic complications is by some considered controversial, even though most studies show an increased risk^{210, 241, 243, 313, 314, 317} as shown in a recent meta-analysis³¹⁸. Poor nutritional state or low preoperative serum albumin have been found in earlier reports to be risk factors for intraabdominal septic complication^{241, 313} together with anemia³¹⁹ and

emergency surgery³²⁰. One of the most convincing factors associated with an increased risk of intra-abdominal septic complications in previous reports is the preoperative intra-abdominal abscess or fistula with a risk of up to 25 %^{241, 243, 244, 277, 313, 321-323}.

The risk for anastomotic complications has been shown to increase with the number of identified preoperative risk factors. Without any risk factor it is 0-5 % rising to 14-30 % with one risk factor, 16-38 % with two risk factors, and as high as 26-100 % if three or four factors are present²⁴¹, 313, 321. In such high risk patients a temporary protective stoma has been proposed^{241, 313, 321}. Surgery has a long term positive effect on health related quality of life^{75, 324, 325} and it seems to be of less importance if remission is achieved through surgery or medical therapy⁸¹. In a study by Scott and Hughes 74 % of patients having had ileocolonic resection would have preferred their surgery in median one year earlier and no patients would have liked the operation later. Patients having repeat surgery were however more content with the timing of their surgery 326. In an interesting study from Australia by Byrne et al colorectal surgeons. gastroenterologists and patients with Crohn's disease were asked to express their preferences regarding ileocolic resection, proctocolectomy and biological therapy. Patients were significantly more willing to have all kinds of surgery, except in the case of a permanent stoma or IPAA, in comparison with gastroenterologists. On the other hand no differences were seen between patients and colorectal surgeons, with one exception; surgeons being more willing to go through proctocolectomy with a permanent stoma³²⁷. This finding of diverging preferences further emphasizes both the value of surgery and medicine as complementary treatment modalities in Crohn's disease, and the value of the patient being evaluated by a multidisciplinary team well familiar with inflammatory bowel disease^{80, 328-330}

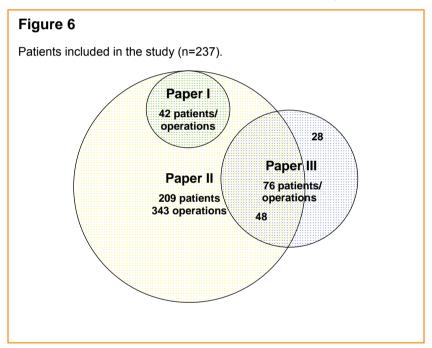
AIMS OF THE STUDY

- I To evaluate the effect of thiopurines in regards to clinical and surgical relapse when given as maintenance therapy after surgical remission in Crohn's disease.
- II To assess whether thiopurines alone, or together with other possible risk factors, are associated with postoperative intraabdominal septic complications in abdominal surgery for Crohn's disease.
- III To investigate whether a split stoma can reduce the number of risk factors and affect the final surgical outcome in high risk patients with ileocolonic Crohn's disease.
- **IV** Investigate the effect of colitis and anti-inflammatory therapies on healing of colonic anastomosis in a mouse model.

PATIENTS AND METHODS

Patients

Since 1989 all patients treated for Crohn's disease at the Linköping University Hospital have been entered into a database for prospective evaluation. The extent of the disease involvement has been registered together with medical and surgical treatments. Moreover, at every visit to the clinic, each patient has assessed their symptoms on a visual analogue scale as well as scoring according to a modified Crohn's Disease Activity Index⁵ (Table 2). The diagnostic criteria established by Lennard-Jones³³¹ and Morson³³² were used for the diagnosis of Crohn's



disease in non-operated and operated patients, respectively. The distribution of patients included in the different papers is presented in Figure 6. In paper I 42 patients were included being considered for post-operative maintenance therapy with azathioprine after going through abdominal resection because of Crohn's disease. In paper II 343 consecutive abdominal operations (in 209 patients) because of Crohn's disease were included and 76 patients were included in paper III. Patients in paper I and II were included regardless of the location of the disease while only patients having ileocecal or ileocolonic resections were included in paper III. For further details regarding the patients studied see paper I-III.

Mice

In paper IV 84 female C57BL/6 mice were bought from HarlanEurope, the Netherlands. After being acclimatised for one week with tap water and standard food ad libitum with a 12 hour light/dark cycle the mice were randomized into receiving either continued tap water or dextran sulfate sodium. At the time of inclusion in the study they weighed 15-22 g.

Methods

Clinical assessment

The signs and symptoms of Crohn's disease were assessed for all patients in paper I-III according to the modification (using only clinical parameters) of the Crohn's Disease Activity Index by Best and Becktel^{1, 5} (Table 2). A symptom score of <150 was considered clinical remission, 150-250 mild activity, 251-400 moderate activity, and >400 as severe disease activity, while a change of 50 points in the index has been classified as a minimal improvement or worsening of the disease¹. In paper I patients also assessed their perceived health on a visual analogue scale where zero is the worst possible score and 100 corresponds to perfect health. Both the modified CDAI as well as the perceived health were integrated over time as the area under the curve.

Medical therapy

A steroid course was considered given when patients received a dose of 10 mg or more of prednisolone (or corresponding dose of another corticosteroid). Regarding preoperative therapy in paper II this was deemed in place if thiopurines had been given for more than three months and within six weeks prior to surgery and if steroids had been given for more than four weeks and within two weeks prior to surgery. Other studies have used less rigid criteria, especially regarding the use of thiopurines. We selected these criteria because of the slow anti-inflammatory onset of thiopurines³⁴⁵, which probably would be associated with a similar slow onset of a potential detrimental effect on the anastomotic healing. Further, there is often a clinical possibility to wean patients off these drugs during 6-8 weeks prior to surgery while optimizing patients^{268, 333}.

Surgical therapy

All surgical procedures included in the papers I-III were because of Crohn's disease. Data regarding operations performed were sequentialy

entered into the database while background data regarding disease location and surgery prior to 1989 were retrospectively entered.

Statistical methods

Values are presented as median and range in paper I, as mean values and standard deviations in paper III, and as median and inter quartile range in paper IV. Comparisons of nominal variables between two groups were made using the Student's t-test (paper III), Mann-Whitney U test (paper I-IV), or permutation test (paper III) while comparisons between several groups were made using analysis of variance (paper III) and Kruskal-Wallis test (paper IV). For comparison of categorized data Chi-square test (paper II-III), Fisher's exact probability test (paper I-II), mid-P exact test (paper III) were used as appropriate. Differences in survival without clinical relapse or repeat abdominal surgery (paper I) were calculated using the Kaplan-Meier and Mantel-Cox log-rank tests. In order to adjust for possible confounding factors in paper III multivariate analysis using logistic regression was used. All P values were two-tailed and P values less than 0.05 were considered significant. Stat-View® statistical package version 5.0.1 (SAS Institute Inc., North Carolina, USA) was used for all statistical analyses.

Table 2

Crohn's Disease Activity Index (CDAI)1

Assessed daily one week prior to visit

$2X_1+5X_2+7X_3+20X_4+30X_5+10X_6+6X_7+X_8$

X₁=Number of liquid stools, sum of seven days rating

X₂=Abdominal pain, sum of seven days rating

0=none, 1=mild, 2=moderate, 3=severe

X₃=General well-being, sum of seven days rating

0=generally well, 1=slightly under par, 2=poor,

3=very poor, 4=terrible

X₄=Extra intestinal complications

Number of listed complications (arthritis/arthralgia,

iritis/uveitis, erythema nodosum, pyoderma gangrenosum, aphtous stomatitis, anal fissure/fistula/abscess.

fever >37.8°C)

X₅=Anti-diarrhoeal use within the previous seven days

0=no, 1=ves

X₆=Abdominal mass

0=no, 2=questionable, 5=definite

X₇=Hematocrit, expected minus observed value

Males=47-observed value

Females=42-observed value

X₈=Body weight, Ideal/observed ratio

[1-(ideal/observed)] x 100

Modified Crohn's Disease Activity Index (modified CDAI)⁵

Assessed the day prior to the visit

$20*(X_1+2*(X_2+X_3+X_4+X_5))$

X₁=Number of soft or liquid stools per day

X₂=Abdominal pain rating

0=well, 1=mild, 2=moderate, 3=severe

X₃=Rating of feeling of well-being

0=well, 1=slightly below par, 2=poor,

3=very poor, 4=terrible

X₄=Number of extra intestinal findings complications (arthritis/arthralgia,

iritis/uveitis, erythema nodosum, pyoderma gangrenosum,

aphtous stomatitis, anal fissure/fistula/abscess,

fever >37.8°C)

X₅=Abdominal mass

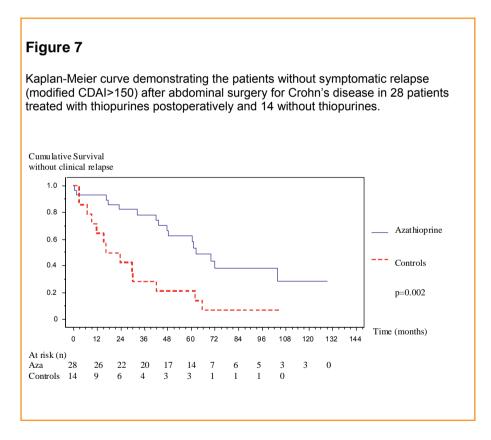
0=none, 2=questionable, 5=present

RESULTS

Detailed descriptions of the results are given in the respective papers. Only important findings are high-lighted in this section.

Paper I

Patients on thiopurines had less symptoms expressed as a lower CDAI over time (CDAI integrated as the area under the curve during the follow up), 100.4 (1.8-280) compared with 161.4 (22.9-370) in the control group (p<0.05). The recurrence rate after two years was 28 % for thiopurine treated patients compared to 50 % for those without thiopurines (ns). Furthermore the patients receiving thiopurines had a longer time to first clinical relapse (p=0.01), 47.9 (0.5-129.0) compared with 26.7 (2.7-105.2) months in the control group.



There was no difference between the groups in perceived health over time. Eighteen patients (64 %) in the thiopurine group needed 23 steroid courses during the follow up compared with 12 (86 %) patients in the

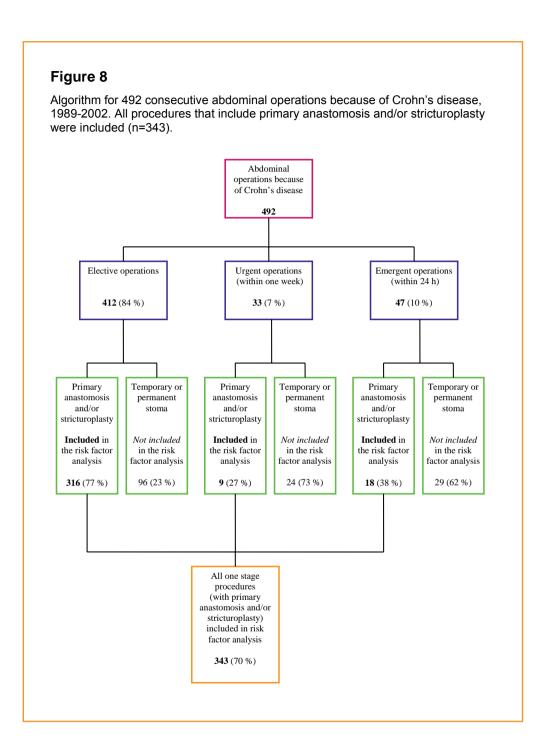
control group needing 30 steroid courses (ns). However, expressed as number of steroid courses per month of follow up the control group had close to the double amount of steroid courses with 0.2 (0.0-1.2) course per month compared to 0.1 (0.0-0.6) course per month for the thiopurine group (p=0.05). Median time to first repeat laparotomy because of Crohn's disease did not differ between the groups, 52.5 (2.8-129.0) compared to 37.1 (11.5-105.2) months. Nor was there a difference in the number of repeated laparotomies per month of follow up, 0.3 (0.1-1.0) for patients on thiopurines and 0.3 (0.1-0.9) for patients without.

Paper II

The thiopurine treatment prior to surgery increased from 9 % during the first seven years of the study period to 19 % during the subsequent seven year period (p=0.01). Patients undergoing primary surgery during the study period received thiopurines before surgery in 4 % of the cases compared to 19 % among those operated earlier (p<0.001).

Postoperative intra-abdominal septic complications occurred in 8 % of the 343 operations studied (Figure 8) and re-intervention within 30 days was needed in 10 % of the operations. In patients treated with thiopurines pre-operatively the risk of postoperative anastomotic complications was increased compared to those without such treatment. 16 % and 6 % respectively (p=0.044). The rate of surgical re-intervention was also increased, 20 % and 9 % respectively (p=0.016). Other risk factors that remained after logistic regression analysis were colo-colonic anastomosis and presence of intra-abdominal fistula or abscess prior to surgery. Anastomotic complications were diagnosed in 16 % after colocolonic anastomosis, in 8 % after entero-enteric anastomosis, in 5 % after stricturoplasty alone, and in 3 % after entero-colonic anastomosis (p=0.031). The presence of a preoperative intra-abdominal fistula or abscess was less frequent among patients receiving an anastomosis but increased the risk of an anastomotic complication from 6 % to 18 % (p=0.024).

Variables like priority for surgery, number or technique of anastomoses, preoperative steroid therapy, high modified CDAI score or previous anastomosis related complications did not significantly increase the rate of anastomosis related complications. However, the group receiving a diverting or permanent stoma had a significantly higher modified CDAI score preoperatively and had more frequently undergone previous abdominal surgery compared to those receiving an anastomosis. In



addition, the patients in the stoma group more frequently received steroids preoperatively, and suffered from hypo-albuminemia (<30 g/l) or anemia preoperatively in comparison to those receiving a primary anastomosis.

Anastomotic complications were diagnosed in 4 % of patients with none of the three risk factors, in 13 % of those with any one risk factor and in 24 % of those with two or three risk factors (Table 3).

Table 3

Correlation between number of risk factors and risk of anastomotic complications after abdominal surgery for Crohn's disease.

Number of risk factors	Number of operations	Number of complications	
None	219 (64 %)	8 (3.7 %)	
Any one	107 (31 %)	14 (13 %)	
Any two	15 (4 %)	3 (20 %)	
All three	2 (1 %)	1 (50 %)	

p<0.0001

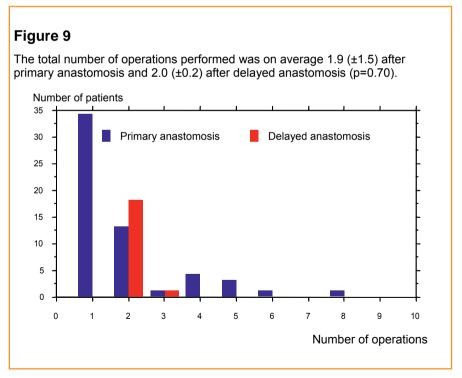
Paper III

Between the years 1995 to 2006 146 operations were performed on 132 patients with ileocolonic Crohn's disease. Seventy six of these patients had two or more preoperative risk factors previously shown to be associated with an increased risk of anastomotic complications. In 19 (25 %) cases patients had been selected to receive a split stoma, where both ends of the future ileo-colonic anastomosis are brought out through a common ostomy opening in the abdominal wall, while the other 57 (75 %) patients received a primary anastomosis.

Of the 15 (12 %) patients who received a primary anastomosis and suffered from anastomotic complications within 30 days 11 patients (73 %) had two or more risk factors. Accordingly the risk for anastomotic complications in the primary anastomosis group was 6 % (4/70) for those with less than two risk factors (low risk group) and 19 % (11/57) for those in the high risk group with two or more risk factors (p=0.018)

The group receiving a split stoma had a significantly higher number of risk factors prior to surgery (p=0.0008), 3.5 (\pm 1.3) compared to 2.4 (\pm 0.7) in the group with primary anastomoses. The differing risk factors were hypo-albuminemia, emergency surgery, steroid therapy, and preoperative abdominal abscess or fistula. The number of risk factors at the time of the delayed anastomosis had decreased to 0.2 (\pm 0.5, p<0.0001).

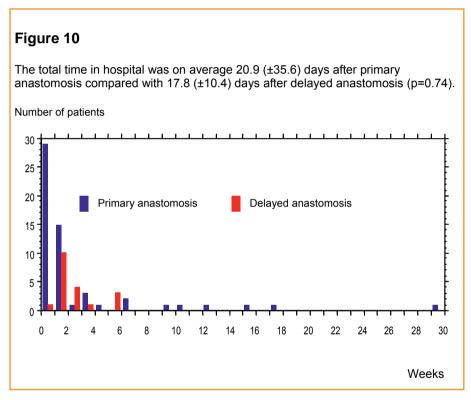
Patients receiving a split stoma had significantly fewer anastomosis related complications (p=0.032) and need of surgical re-intervention (p=0.032) within 30 days of surgery, 0 (0 %) compared to 11 (19 %) respectively for both types of complications. In the stoma group the number of complications was combined for both the primary resection and the delayed anastomosis. The patients receiving a stoma had more problems associated with the stoma (p=0.0002) than in the primary anastomosis group in general, 9 (47 %) compared with 5 (9 %). This difference disappeared when compared only to those eleven in the primary anastomosis group who actually received a stoma (due to complications), 5 (45 %, p=0.92).



Three patients presented with late anastomotic complications up to three months after a primary or delayed anastomosis, giving a total frequency of anastomotic complications of 23 % (13/57) and 5 % (1/19)

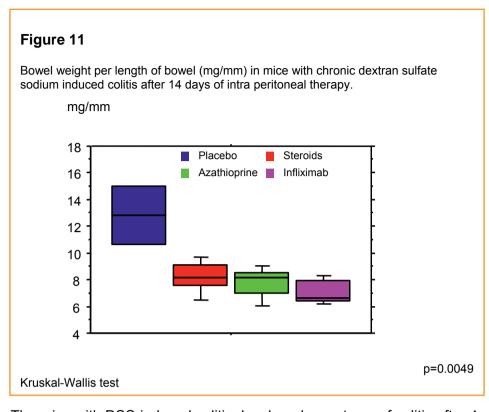
respectively (p=0.090). All these patients had two or more risk factors at the time of primary (n=2) or delayed anastomosis (n=1).

The time span until the split stoma was closed and a delayed anastomosis performed was in median 5.0 (2.3-12.6) months. Six months after the primary surgery 58 % (11/19) had their bowel continuity restored and 79 % (15/19) after 9 months. There were no differences between the two groups regarding the total number of operations performed (Figure 9) or the total time in hospital (Figure 10).



Paper IV

Eighty-four C57BL6 mice were randomly divided into two groups, 40 receiving tap water and 44 receiving 3 % DSS for five days. The two groups were then further randomly divided into four groups each, receiving placebo, prednisolone (2 mg/kg bodyweight), azathioprine (5 mg/kg bodyweight) or infliximab (5 mg/kg bodyweight) during 14 days after which surgery with a colo-colonic anastomosis was performed. After 48-52 hours postoperatively the mice were sacrificed and anastomotic healing was assessed by bursting pressure.



The mice with DSS-induced colitis developed symptoms of colitis after 4-7 days and lost weight compared to the tap water group. Bowel weight per length of bowel (mg/mm) is used to describe the severity of colitis and differed significantly between the tap water group compared to the DSS group, 5.3 (4.7-6.1) mg/mm compared to 8.1 (6.9-8.8) mg/mm (p<0.0001). In the DSS group the mice receiving placebo had a more active inflammation (Figure 11) with a value of 12.8 (10.6-15.0) mg/mm, which differed significantly from all the other therapy arms; prednisolone 8.1 (7.5-9.1) mg/mm (p=0.014), azathioprine 8.2 (7.0-8.5) mg/mm (p=0.0046), infliximab 6.7 (6.4-7.9) mg/mm (p=0.0055).

The median bursting pressure for all mice surviving colitis and surgery was 81.0 (62.3-104.3) mmHg. The bursting pressure in the placebo group did not differ from the azathioprine or infliximab groups (Table 4). In contrast bursting pressure for the group receiving prednisolone differed from placebo with a decreased bursting pressure. The bursting pressure in the prednisolone group was also decreased in comparison to azathioprine (p=0.0004) as well as infliximab (p=0.0015).

Table 4

Bursting pressure (mmHg) of colo-colonic anastomosis after 48-52 hours in mice with/without dextran sulfate sodium induced colitis.

Placebo	Prednisolone	Azathioprine	Infliximab
90.0 (71.5-102.8)	55.5 (42.8-73.0) p=0.0004*	84.4 (70.5-112.5) p=0.93*	92.3 (75.8-122.3) p=0.45*

p=0.0021[‡]

Median values and inter-quartile range.

[‡]Kruskal-Wallis test.

^{*}Statistical analyses in comparison with placebo (Mann-Whitney *U*-test).

DISCUSSION

This thesis is focused on the combined use of surgery and medicine as complementary modalities in the treatment of Crohn's disease.

When thiopurines were given as postoperative maintenance treatment, patients tolerable to the drug had a more favorable course than those intolerant to the drug but otherwise equal in disease severity. Thiopurines given as postoperative maintenance treatment further reduced symptoms and prolonged time to symptomatic relapse. Moreover, the thiopurine-treated patients needed less corticosteroids, similar to the data presented by Holtmann *et al*³³⁴.

Our study-patients consisted of a subgroup with aggressive disease, comprising less than 20 % of our patient population. The study was retrospective in its design but based on prospectively gathered data and all patients were selected to receive thiopurine therapy. Furthermore, 86 % of the patients had been receiving the targeted daily azathioprine dose of 2.0-2.5 mg/kg and no one received a lower dose than 1.25 mg/kg. Thiopurines decreased the relapse rate after two years from 50 to 28 %, which is similar to figures in a randomized controlled study from Hanauer and co-workers who reported a decrease from 77 to 50 %for placebo and thiopurine respectively²⁷⁴. They did however not find a difference between thiopurine and mesalazine, which may be due to their use of a relatively low and standardized dose of 50 mg 6mercaptopurine daily, approximately corresponding to 100 mg azathioprine daily³³⁵. Their study comprised five tertiary referral centers and a relapse rate of 77 % in non-treated patients is high, indicating that they treated a cohort with very aggressive disease.

Ardizzone and co-workers were unable to demonstrate any benefits with thiopurines compared to mesalamine 336. Their relapse rates after two years were 28 and 17 % for thiopurines and mesalamine respectively, indicating that their study comprised patients with less aggressive disease. However, they did find thiopurines to be beneficial in previously resected patients, presumably with a more aggressive disease compared to previously non-resected patients.

The most recent study on thiopurines post-operatively was published by D'Haens *et al* in Gastroenterology last year. They found a positive effect on the endoscopic recurrance rate one year after surgery, in patients at high risk of early recurrance, using a combination of antibiotics and thiopurines with azathioprine given for one year and metronidazole or placebo added during the first three months¹⁷³. The combination of

azathioprine and metronidazole significantly decreased the recurrence rate from 78 % to 55 % after 12 months. Taken together, these data point to that thiopurines given as postoperative maintenance treatment are effective in delaying clinical relapse in patients with aggressive Crohn's disease.

No differences were found in need of repeated abdominal surgery, which in all cases except two were due to re-stricturing of the intestine. Our series is most likely too small to detect any favorable effect of thiopurines and to proove a non-effect a considerably larger number of patients are needed. However, Ardizzone et al had similar findings³³⁶ and also an uncontrolled study from Korelitz and co-workers found that thiopurines did not eliminate the need of repeat surgery for small bowel obstruction¹⁷⁷. Cosnes et al studied the need of surgery during two time periods and found no impact on the need of surgery by the use of thiopurines during the latter time period³³⁷. A similar finding was found in a systematic review by Wolters et al where no changes were seen over a period of four decades in regards to surgical recurrences after primary resection³³⁸. A recent study on 170 primary ileo-cecal resections found a protective effect on the rate of repeat ileo-colic resections after 15 years with rates of 40 % among those on immunomodulators compared to 80 % among those without (p=0.022)⁹⁸. Some have advocated discontinuing thiopurine medication in patients in complete remission without steroids^{334, 339} but a number of reports have shown withdrawal of thiopurines to be associated with an increased risk of flares 186, 340, 341 but not with an increased risk of repeat surgery³⁴². Nor is there any difference in regard to surgery among patients on scheduled maintenance therapy with infliximab whether thiopurines are added or not³⁴³. In an evaluation of 30 years of thiopurine use at the Oxford IBDclinic immuno modulation was safe and effective but as many as 25 % of the patients with Crohn's disease were in need of surgery during the first six months¹⁷⁵. All together this might implicate that thiopurines, in spite of reducing symptoms and the need of steroids, may be less effective in preventing submucosal fibrosis and formation of intestinal strictures.

Earlier this year the first report on the use of biologics in the postoperative setting was published. In a study on 24 patients undergoing ileocolonic resection for Crohn's disease infliximab was given within four weeks post-operatively and continued for one year. This regimen was found to have a positive effect on the number of endoscopic and histological recurrences but no effect on the number of clinical relapses compared to placebo³⁴⁴. When patients become in need of surgery we have paid attention to established preoperative risk factors (e.g. steroid therapy) with a cautious attitude and refrained from primary anastomosis in 30 % of the operations. For example, only 25 % of the patients receiving an anastomosis were on corticosteroids compared to 83 % in the group receiving a stoma. This may have contributed to a complication rate of 8 % of the operations with an anastomosis, rates well in line with previous reports from other referral centers for inflammatory bowel disease 211, 241, 243, 321

We found that preoperative immunomodulation with thiopurines was significantly associated with an increased rate of anastomotic complications of 16 % compared to 6 % for patients without immunomodulation (p=0.044). Possible reasons for an impaired anastomotic healing with immunomodulation with thiopurines include Tcell-mediated suppression, decreased proliferation and increased apoptosis of epithelial cells^{208, 345}. At the same time thiopurines have a steroid sparing effect³⁴⁶ and the adverse effect of steroids on wound healing might be of greater importance than a possible adverse effect of immuno modulation with thiopurines^{210, 241, 243, 321}. There are five other reports regarding the effect of thiopurines on postoperative recovery but these are either on a mixture of patients with ulcerative colitis and Crohn's disease, with concomitant corticosteroid therapy or complications not separated into anastomotic complications and general infectious complications^{210-212, 347, 348}. Our data is prospectively gathered, strictly on patients with Crohn's disease undergoing abdominal surgery with one or more unprotected anastomoses and/or stricturoplasties, and focused on anastomotic complications - surgically the most severe complication. Further, compared to the other reports we have a relatively low rate of steroid treated patients (especially on high dose regimens) which may cause other risk factors for anastomotic complications to become more evident^{210-212, 347, 348}. The knowledge of how long the thiopurine effect is sustained after withdrawal is scarce. In our study we selected six weeks to be the cut off period prior to surgery as it is a time period possible to use clinically similar to the tapering of steroids. Further, we demanded the thiopurine therapy to have been ongoing for at least three months as it acts slowly and a possible clinical effect can be evaluated ≥17 weeks after induction of remission in active disease³⁴⁹.

Aberra *et al* retrospectively studied 159 patients with ulcerative colitis or Crohn's disease, with elective abdominal surgery, with or without anastomosis, excluding all patients with pre-operative intra-abdominal septic complications and pyogenic complications of the disease²¹⁰. The rate of major infectious complications was 20% for steroid treated patients, 12% for thiopurines with (n=34) or without (n=18) steroids, and

4 % for those without any of these treatments (n=51). Although the difference was not statistically significant, the rates are similar to our data. However, the complications were analyzed according to minor (urinary tract infection and temperature >38.6°C) or major complications (e.g. wound infection, sepsis, pneumonia, peritonitis, abdominal abscess, and wound dehiscence) and not specified for postoperative intra-abdominal septic complications, by far the most feared complication. In a study of 270 Crohn's disease patients by Colombel and coworkers³¹⁵, 26% received a stoma and 74% received stricturoplasty or one or more anastomoses. In that study, there was an obvious risk of selection bias when later analyzing all of the patients together, since high-risk patients were more likely to receive a stoma. No adverse effect was seen in the group receiving immuno modulation, but most of the patients were treated with steroids and only eight patients (3 %) received immuno modulators alone and 33 patients (12 %) received no medication prior to surgery. Furthermore, some of the patients received their immuno modulating therapy for less than two weeks prior to surgery, a time period that probably is too short to cause any negative effect³⁴⁹ and the reason why we selected a longer treatment period. At the same time patients who terminated their immuno modulation as late as four weeks prior to surgery were coded as not on immuno modulation, a questionably short wash out period. Our study emphasizes anastomotic complications, the most severe complication. whereas Colombel et al included these in a larger group of septic complications (e.g. wound infection, pneumonia, sepsis, bacteraemia, and anastomotic complications); the 5% who developed anastomotic complications were not further analyzed. In the third study by Tay et al they propose an improved perioperative outcome after immuno modulation in 100 studied patients with Crohn's disease undergoing primary surgery with anastomosis or stricturoplasty³⁴⁷. Immuno modulators were analysed together with biologics and further data regarding medications as well as the patients are limited in the report making it hard to evaluate. In a recent study from the Mayo Clinic on ileal pouch anal-anastomoses in ulcerative colitis an increased risk of infectious complications (anastomotic leak, pelvic abscess, or wound infection) was seen in patients pre-operatively treated with thiopurines in a univariate analysis (odds ratio=2.1; 95% CI, 1.1-4.3), but not after a multiple variable logistic regression analysis (odds ratio=1.3; 95 % CI, 0.6-2.9)³⁴⁸. Mahadevan et al studied the early outcome of ileal pouchanal anastomosis in 209 patients with ulcerative colitis³¹⁶. Sixteen percent (9/55) of the patients on thiopurines had their immuno modulating therapy withdrawn as late as one week before surgery and were coded as not on immuno modulation. Among the remaining 46 patients with thiopurines, the rate of anastomotic complication was 9 %

compared to 6 % in those without thiopurines (not significantly different). In their study, high-dose steroids and severe or fulminant disease according to Truelove-Witt factors correlated with an increased risk of anastomotic complications (p<0.01).

We had expected that high disease activity would increase the complication rate but in multivariate analysis the modified CDAI was of no consequence, probably because patients with high activity index were operated with a stoma rather than an anastomosis. Moreover. there is poor correlation between CDAI and the degree of local mucosal inflammation. Thiopurine therapy might also be a confounder in making the disease less severe and with a lower symptomatic load than without immunomodulation³⁴⁶. In agreement with the findings of others we found an increased risk of anastomotic complications in the presence of preoperative fistula and/or abscess²⁴¹⁻²⁴⁴ and for colo-colonic anastomoses compared with other sites of anastomosis 321, 350. Poor nutritional state or low preoperative serum albumin has been found to be risk factors for anastomotic complications^{241, 321} and in our material a similar strong tendency towards an increased risk was found among patients with low serum albumin preoperatively. The indications for surgery, if it was urgent or elective, number of previous laparotomies, as well as the number of anastomoses did not increase the risk, nor did an earlier history of anastomotic complications affect the outcome. Patients operated more than once during the study are prone to suffer from a more aggressive disease and also received thiopurines more frequently. The high rate of stomas in favor of anastomoses in this group may explain why no increase was seen in the rate of anastomotic complications.

With the increasing use of biologics in inflammatory bowel disease the worry regarding a possible affect on the surgical outcome has arisen again. In the previously mentioned study by Colombel *et al* in patients with Crohn's disease³¹⁵ no increase was seen among the 52 patients receiving infliximab prior to surgery. The same result was found in two other reports^{351, 352}. Marchal *et al* who compared 40 patients with Crohn's disease who received infliximab prior to intestinal resection to 39 matched controls of infliximab naïve patients³⁵². A trend was seen towards an increased early infection rate among patients on biologics but these patients also received steroids and immuno modulating treatment in a significantly higher degree. The opposite finding was found in the study by Kunitake *et al* who found a decreased incidence of infectious complications in patients with inflammatory bowel disease on infliximab compared to those without, despite the infliximab patients also were receiving immuno modulators in a higher rate³⁵¹. One limitation in

this report is a significantly higher frequency of pre-operative intraabdominal sepsis in the non-infliximab group, 10.9 % compared with 4.0% (p=0.036). In the same issue the Cleveland Clinic reports an increased risk of post-operative sepsis (p=0.027), intra-abdominal abscess (p=0.005), and readmissions within 30 days (p=0.045) in patients with Crohn's disease receiving infliximab within three months prior to ileocolonic resection³⁵³. The same group found similar risks after proctocolectomy with ileal pouch anal-anastomoses in chronic ulcerative colitis with more than twice the number of overall early post-operative complications (p=0.027)³⁵⁴. This was especially seen in the aspect of postoperative sepsis (p=0.016) and anastomotic leaks (p=0.023). increasing from 2.2 % to 21.7 % and 17.4 % respectively. A report from the Mayo Clinic had similar findings with increased risk of infectious complications in infliximab treated patients with an odds ratio of 3.5 (95 % CI 1.6-7.5)³⁴⁸. These reports are in contrast with the report by Schluender et al who found no increased risk in the same patient group. except for a small subgroup of patients (n=5) receiving concomitant treatment with infliximab and cyclosporine A who had a 80 % complication rate (p=0.04)³⁵⁵. In another study from Oxford steroids were compared to the combination of steroids and cyclosporine A in patients with ulcerative colitis undergoing colectomy and ileostomy³⁵⁶. No differences were seen between the groups but a 20 % major surgical complication rate was seen, pointing to a severely ill group of patients.

To verify if biologics and/or immuno modulators are interfering with the healing capacities or not requires large randomised multi-centre studies. Maybe centres showing adverse effects of these therapies are operating patients at a later stage of the disease compared to other centres not finding increased risks³⁵⁷. Thus these treatments may only act as surrogate markers for patients with a more severe illness, with an inherent healing disturbance or at least more prone to be associated with an increased risk of post-operative complications. Data pointing in this direction are the reports from Scarpa *et al* and Welsch *et al* where patients with Crohn's disease suffering from postoperative complications had an increased risk of early surgical recurrences (p=0.008 and p=0.0006 respectively)^{266, 267}, warranting prophylactic thiopurine use according to the authors²⁶⁶. This increased risk of early relapsing of the disease after a surgical complication may be the other way around; the early relapse is in fact a signal for an aggressive disease which is associated with an increased risk of post-operative complications.

When different immuno modulators have been tried in animal models they have showed different results, sometimes contradictory results, and they have as far as we know never been tested in comparison with an inflammatory condition. A strong point with our study is this comparison of colitis/no colitis and anti-inflammatory therapy/no anti-inflammatory therapy on the healing of colonic anastomoses, trying to evaluate if the actual risk factor is the therapy or the inflammatory disease itself. The anti inflammatory effects on the DSS-induced colitis (less weight per length of colon) of all therapies compared with placebo speaks in favour of the used doses and interval to being clinically significant.

Just as shown in paper IV corticosteroids have an adverse effect on the healing of bowel anastomoses in regards to obvious leaks as well as decreased bursting pressures³⁵⁸⁻³⁶¹. Possible reasons for this effect is inhibition of collagen synthesis, through blocking of TGF-β and/or intercellular adhesion molecule-1 (ICAM-1)^{358, 359, 362, 363}. In dogs, Lima *et* al found twice the breaking strength in bronchial anastomoses and four times the breaking strength in skin among controls compared to dogs treated with a combination of prednisolone (2 mg/kg) and azathioprine (1.5 mg/kg)³⁶⁴. However when giving only azathioprine we could not find any adverse effect on the bursting pressure. Nor did Stolzenburg et al. who tested the anastomotic healing using breaking strength, with up to four times as high dose of azathioprine as in our study³⁶⁵. The use of tacrolimus has only been tested in two studies; with detrimental effect on dermal healing^{366, 367}. In one of the studies they also studied the bursting pressure in colonic anastomoses without any influence of tacrolimus in comparison with controls, in contrast to the effect on dermal healing³⁶⁶. Cyclosporine A has only been evaluated through breaking strength of dermal incisions in rats and was found to impair the breaking strength and accumulation of collagen in the wound compared to controls^{368, 369}. Mycophenolate mofetil (MMF) has been studied by bursting pressure of colonic anastomoses in rats with a 40 % and 33 % reduction on postoperative days 2 and 4 respectively³⁷⁰. No effect was however seen on the synthesis of collagen in contrast to the effect of steroids. Instead, a decrease in acidic mucins was seen in MMF-treated animals, postulating a possible diminished protection of the healing process from luminal pathogens. An interesting finding on the association between anastomotic healing and acidic mucins is the report by Egger et al 371. Systemic keratinocyte growth factor (KGF) was administered to rats and was found to increase the bursting pressure by 19-49 % during the first seven days postoperatively (p<0.05). At the same time an increase of the colonic crypt depth and acidic mucin content at the anastomosis (p<0.05) was found but no difference in collagen deposition, promoting a possible role of the mucus in protecting the healing of a colonic anastomosis. Moreover, KGF has been found to ameliorate the inflammation in DSS-induced colitis in mice as well as TNBS-induced colitis in rats^{372, 373}. Another interesting finding is the effect of

doxycycline, an antibiotic used as preoperative prophylaxis prior to colorectal surgery at our unit. It is known to inhibit the MMP-activity and even increase the colonic bursting pressure and breaking strength in rats by 93 % and 27 % (p=0.0002 and p=0.0019 respectively) when given one day preoperatively and until sacrifice 1, 3 or 5 days postoperatively 374 .

The collagen synthesis seems to be of importance in the healing of an anastomosis but may also be involved in the stricturing of the inflamed bowel segment. Histologic studies have shown both muscle layers as well as the submucosa being involved in the stricturing of the bowel³⁷⁵. The muscle layers are expanded by a combination of proliferation and hypertrophy while the submucosa is expanded by accumulation of collagen, especially type III and V collagen similar to atherosclerosis 376-³⁷⁸. Transforming growth factor-β (TGF-β) augments the collagen synthesis in human intestinal smooth muscle. Together with platelet derived growth factor (PDGF) and interleukin-1β (IL-1β) TGF-β plays an important role in the healing of tissue in response to injuries like atherosclerosis and inflammation 377, 379, 380. An in vitro study with regenerating agents (dextrans mimicking the growth factor effects of heparin sulphates) showed an ability to decrease total collagen production by 50 %, 76 % of collagen type III but almost no effect on type V³⁸¹ but need further evaluation regarding a possible effect on diminishing the risk of stricturing by the disease.

Matrix metalloproteinases (MMP) are elevated in both ulcerative colitis (inflamed mucosa) and Crohn's disease (inflamed and non-inflamed mucosa), especially MMP-1, -3 and -9^{379, 380, 382-384}. Di Sabatino et al showed in a report from this year that TGF-β together with tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) is elevated in the mucosa above Crohn strictures compared with the mucosa of nonstrictured gut while MMP-3 and -12 are elevated in inflamed mucosa but reduced in strictures³⁸⁵, in contrast to a previous report³⁸⁶. With the aim of preventing stricturing of the gut in Crohn's disease the pharmacological effects on the expression of different MMP has started to be evaluated. The anti-tumor necrosis factor-α antibody infliximab has been shown to down-regulate the expression of MMP-1, -3 and -9 in the intestine $^{\rm 387,\,388}$ and of MMP-9 in serum while MMP-2 was increased in serum³⁸⁸. However, high levels of MMP-9 and TIMP-1 in non-inflamed mucosa from resected Crohn patients has been shown to be associated with a favorable outcome in regards to clinical as well as surgical recurrence³⁸⁹.

There might be a vicious circle between stricturing and inflammation in the intestine as seen in the example of ameliorating effect on the inflammation by stricturoplasty²⁹². The stenosis is causing the enteric content to stand under high pressure, altering the permeability and presumably triggering the mucosal immune system²⁹². This might explainin the different findings regarding MMP in patients with Crohn's disease and maybe why stricturoplasty seems to be a safe procedure even though it is performed in an inflamed area. In animal models broad inhibition of MMP has shown an ameliorating effect on trinitrobenzensulfonic acid (TNBS) induced colitis 390, 391. Further, such inhibition has shown increased bursting pressure by 28-48 % in colonic anastomoses in rats^{392, 393} opening up an interesting field of antiinflammatory therapy as well as attempts at decreasing the surgical risks in Crohn's disease. There were however no signs of a superior effect on the bursting pressure by any of the anti inflammatory therapies given in our model in comparison with placebo.

Concerning the effect of infliximab on the healing of colonic anastomosis our study is to our knowledge the only one and did not show any adverse effects at all. In one study evaluating treatment with a TNF-binding protein and its effect on healing of the skin showed a decrease by 50 % in the breaking of the wound in animals with repeated injections (p<0.05) while no difference was seen in animals given a single dose pre-operatively³⁹⁴.

In line with our results previous reports have found a correlation between the number of pre-operative risk factors and the risk of developing post-operative anastomotic complications^{241, 321}. Anastomotic breakdown, often followed by a number of repeated laparotomies in order to control sepsis, is the most common cause of surgically induced intestinal failure in Crohn's disease³¹. It may sometimes lead to short bowel syndrome, with detrimental impact on quality of life^{395, 396} or increasing the risk for early relapse of the disease²⁶⁶⁻²⁶⁸. It is therefore of utmost importance to perform as safe surgery as possible in complicated Crohn's disease. In order to decrease the risk of anastomotic complications in high risk patients it has often been proposed to protect the anastomosis with a proximal defunctioning stoma or even to only deviate with a delayed resection and anastomosis^{241, 313, 317, 321, 353, 397, 398} if not possible to reduce the number of risk factors and optimize the patient preoperatively^{268, 333}.

We used a split stoma, previously described as an anastomotic stoma^{399,} with both ends of the future anastomosis brought out through a common ostomy opening in the abdominal wall. This was found to

reduce and even to eliminate the number of preoperative risk factors in cases of severe Crohn's disease. We chose the split stoma in patients at high risk of anastomotic complications in favour of a primary anastomosis with a proximal diverting stoma. A split stoma has potential advantages compared to a diverting stoma proximal to a high risk anastomosis. Firstly, we do not open up a disease free part of the bowel to construct the stoma, thus creating only one suture line to heal instead of two with an inherent risk of complications. Secondly, we do not perform an anastomosis until the patient is in an optimised condition for healing. Thirdly, studies on animals have in fact shown delayed healing with decreased bursting pressure and decreased collagen content in colonic anastomoses protected by a proximal diversion compared with unprotected anastomoses⁴⁰¹⁻⁴⁰³. This finding points in favour of the importance of the faecal stream and a normal passage of nutrients and bacteria through the anastomosis. Short-chain fatty acids, produced by bacterial fermentation of dietary fibres, have been shown to improve the healing of experimental colonic anastomoses in animals⁴⁰⁴ while germ free rats have a significantly lower bursting pressure 405.

Delayed anastomosis in high risk surgery for Crohn's disease is consequently a safe procedure^{399, 400} with less risk of early anastomotic complications than a primary anastomosis, without adding either a prolonged hospital stay or an increased number of operations.

CONCLUSIONS

- Postoperative maintenance thiopurine therapy seems to prolong the time to clinical relapse, ameliorating the symptoms over time with a steroid sparing effect.
- Preoperative thiopurine therapy, colo-colonic anastomoses and preoperative intra-abdominal sepsis were found to be associated with an increased risk of anastomotic complications in abdominal surgery for Crohn's disease.
- An increasing number of preoperative risk factors were associated with a significantly increasing risk of anastomotic complications.
- The detrimental effect of corticosteroids on the healing of anastomoses measured through bursting pressure was verified in an animal model.
- Azathioprine or infliximab therapies were found not to influence the anastomotic healing of a colo-colonic anastomosis in the same animal model.
- The use of split stomas in selected cases of high risk patients with Crohn's disease decreases the number of risk factors prior to the anastomosis, with less risk of early anastomotic complications and without increasing the number of surgical procedures or the time spent in hospital.

CLINICAL APPLICATIONS OF THE THESIS

In the preoperative assessment of patients with Crohn's disease a careful evaluation of the number of risk factors is vital. Most patients will have no or only a single known risk factor prior to surgery and can have a primary anastomosis without increased risk of complications. Patients with two or more known risk factors (e.g. steroids, preoperative intra abdominal infection, need of immuno modulating therapy, poor nutritional state, hypo-albuminemia, anemia) prior to abdominal surgery are however at high risk of post operative anastomotic complications. The risk of severe complications increases with an increasing number of risk factors (Paper II).

If it is not possible to optimize the patient and decrease the number of risk factors prior to surgery (e.g. by tapering of steroids, drainage of abscesses and nutritional support) one should consider refraining from a primary anastomosis or protecting it with a proximal diverting stoma. Another possibility is to perform a split stoma, bringing out both ends of the future anastomosis through the same stoma opening in the abdominal wall. When the patient has recovered and the number of risk factors has decreased a delayed anastomosis can be performed after 3-4 months through the stoma opening. This two stage procedure carries less risk of early anastomotic complications, without adding to either the number of surgical procedures or time spent in hospital (Paper III).

Steroids have been shown to have a detrimental effect on the healing of anastomoses, clinically as well as in animal models. Neither immuno modulation with thiopurines nor biological therapy with infliximab were found to impair the healing of colonic anastomoses in mice measured through bursting pressure (Paper IV). The clinical experiences on both thiopurines and infliximab in surgery for inflammatory bowel disease divert. A possible reason for this disparity is a subset of patients with a more aggressive disease form that is more prone to be affected by surgical complications as well as receiving more active pharmacological therapy.

In patients with a more aggressive Crohn's disease (e.g. young age at onset, smoking, repeat resection, steroid use, intra-abdominal abscess or fistula at surgery), at high risk of early post-operative recurrences, one should consider putting the patients on post-operative thiopurine maintenance therapy (Paper I). Moreover, in patients who are thiopurine naïve one should also consider the use of antibiotic therapy with metronidazole or ornidazole during the three month onset period of the thiopurines. The use of biologics in the post-operative setting needs further evaluation.

SVENSK SAMMANFATTNING

Crohns sjukdom är en kronisk inflammatorisk tarmsjukdom av oklar orsak. Huvudsyftet med denna avhandling var att undersöka den kombinerade behandlingen med kirurgi och immunhämmare vid Crohns sjukdom.

Utfallet av medicinsk och kirurgisk behandling vid 371 operationer på 237 patienter mellan 1989 och 2006 utvärderades. Därutöver studerades effekterna av kortison, immunhämmare och behandling med inflammationsdämpande antikroppar på läkning av tjocktarms-skarv på 84 möss med eller utan inflammation i tarmen.

Vid utvalda fall med svårare form av Crohns sjukdom visade sig förebyggande behandling med immunhämmare efter kirurgi förlänga tiden till återfall av symptom från 24 till 53 månader. Patienter med immunhämmare som underhållsbehandling hade också minskade symptom under uppföljningstiden med ett minskat behov av kortison.

Immunhämmande behandling inför kirurgi visade sig, liksom pågående infektion i bukhålan och sydd skarv på tjocktarmen, vara en riskfaktor för att drabbas av komplikationer vid bukkirurgi på grund av Crohns sjukdom. Risken för infektionskomplikationer i bukhålan ökade från 4 % hos dem utan någon av dessa riskfaktorer till 13 % hos dem med någon och 24 % hos dem med två eller tre riskfaktorer inför operationen. Hos patienter med två eller fler kända riskfaktorer bör man överväga att avstå från att sy en skarv på tarmen vid kirurgi eller möjligen skydda skarven med en avlastande stomi. Ett alternativ till detta är att anlägga en delad stomi där bägge ändarna av den framtida skarven tas ut genom en och samma stomiöppning i bukväggen. Denna metod med en fördröjd skarv på tarmen visade sig minska antalet kirurgiska riskfaktorer inför själva skarvningen och dessutom minska risken för tidiga infektiösa komplikationer i bukhålan, utan att vare sig öka antalet kirurgiska ingrepp eller förlänga vårdtiden på sjukhus.

I en djurmodell visade sig alla tre läkemedlen ha en lindrande effekt på tarminflammation jämfört med placebo. Endast kortison visade sig påverka läkningen negativt med en sänkning av bristningstrycket i den sydda skarven på tjocktarmen, jämfört med placebo såväl som med immunhämmare och antikropps-behandling. Kopplingen mellan immunhämmare och komplikationer efter sydda skarvar på tarmen behöver alltså inte vara en direkt läkemedelseffekt. Orsaken kan istället vara att en undergrupp av Crohnpatienter har en svårare sjukdomsform som ger både ökad komplikationsrisk och större behov av intensiv medicinsk behandling.

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