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**INFLAMMATION IN THE ILEAL POUCH: ASSOCIATED  
FACTORS AND LONG-TERM PROGNOSIS**

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Academic Dissertation

*To be publicly discussed, by permission of the Medical Faculty of the  
University of Helsinki, in Auditorium 2 of the Biomedicum,  
Haartmaninkatu 8, on January 23, 2004, at 12 noon*

Helsinki 2004

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ISBN 952-91-6750-4 (Print)

ISBN 952-10-1574-8 (PDF, <http://ethesis.helsinki.fi>)

Helsinki 2003, Helsinki University Printing House

*To Raili, Joonas, and Julia*



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## LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications, referred to in the text by the Roman numerals I-V.

- I Kuisma J, Nuutinen H, Luukkonen P, Järvinen H, Kahri A, Färkkilä M. Long-term metabolic consequences of ileal pouch-anal anastomosis for ulcerative colitis. *Am J Gastroenterol* 2001;96:3110-3116.
- II Kuisma J, Luukkonen P, Järvinen H, Kahri A, Färkkilä M. Risk of osteopenia after proctocolectomy and ileal pouch-anal anastomosis for ulcerative colitis. *Scand J Gastroenterol* 2002;37:171-176.
- III Kuisma J, Mentula S, Järvinen H, Kahri A, Saxelin M, Färkkilä M. Effect of *Lactobacillus rhamnosus* GG on ileal-pouch inflammation and microbial flora. *Aliment Pharmacol Ther* 2003;17:509-515.
- IV Kuisma J, Mentula S, Luukkonen P, Järvinen H, Kahri A, Färkkilä M. Factors associated with ileal mucosal morphology and inflammation in patients with ileal pouch-anal anastomosis for ulcerative colitis. *Dis Colon Rectum* 2003;46:1476-1483.
- V Kuisma J, Järvinen H, Kahri H, Färkkilä M. Factors associated with pouchitis disease activity after surgery for ulcerative colitis. Submitted for publication.

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## ABBREVIATIONS

AS	Ancylosing spondylitis
5-ASA	5-aminosalicylic acid
AZA	Azathioprine
b.i.d	Twice a day
BMI	Body mass index
BMD	Bone mineral density
CA	Cholic acid
CDCA	Chenodeoxycholic acid
CFU	Colony-forming unit
EIM	Extraintestinal manifestation
F/U	Follow-up
GALT	Gut-associated lymphoid tissue
H & E	Haematoxylin and eosin stain
HID-AB	High iron diamin-alcian blue stain
HDL	High-density lipoprotein
HLA	Human leukocyte antigen
IBD	Inflammatory bowel disease
IL-1RA	Interleukin-1 receptor antagonist
IPAA	Ileal pouch-anal anastomosis
IPS	Irritable pouch syndrome
IS	Conventional ileostomy
LDL	Low-density lipoprotein
LGG	<i>Lactobacillus</i> GG
MHC	Major histocompatibility complex
NO	Nitric oxide
pANCA	Perinuclear antineutrophil cytoplasmic antibody
PAS	Pouchitis activity score
PDAI	Pouchitis disease activity index
PSC	Primary sclerosing cholangitis
PTH	Parathyroid hormone
SCFA	Short-chain fatty acid
SeHCAT	Selenium homocholic acid test
SEM	Standard error of the mean
TNF	Tumor necrosis factor
UC	Ulcerative colitis
U-NTX	Urinary N-telopeptide cross-linked collagen-type

## ABSTRACT

**Background:** Pouchitis, the main complication after ileal pouch-anal anastomosis, occurs in 30 to 59% of the patients undergoing such surgery for UC. The aetiology of pouchitis is probably a multifactorial event involving genetic, immune, microbial, and toxic mediators. Inflammation in the ileal pouch leads to changes in mucosal morphology, with consequent decreased vitamin B<sub>12</sub>, bile acid, and cholesterol absorption documented. This study was undertaken to evaluate possible factors predictive for inflammation activity and mucosal morphology, to evaluate long-term metabolic consequences, and, because manipulating the intestinal microflora by probiotics may prevent pouchitis, to evaluate the effect of *Lactobacillus rhamnosus* GG supplementation as the primary therapy for ileal-pouch inflammation. Finally, to study quality of life at least 5 years after ileal pouch-anal anastomosis for ulcerative colitis.

**Patients and methods:** Subjects eligible for study participation were those (n= 241), who had undergone restorative proctocolectomy with an ileal pouch-anal anastomosis (IPAA) for ulcerative colitis between 1985 and 1994 at the Department of Surgery at Helsinki University Central Hospital. The original study population comprised 128 study subjects: 107 with ileal pouch-anal anastomosis and 21 ileostomy controls. Routine blood tests were run, and vitamin status, bile acid absorption, and bone mineral density were determined, and endoscopy was done, with biopsies. A pouchitis disease-activity index, PDAI, was calculated. Fresh faecal samples and biopsies taken from the pouch and afferent limb underwent bacterial culture before and after *Lactobacillus rhamnosus* GG supplementation.

**Main results:** After a mean 7.5-year follow-up, the cumulative incidence of pouchitis was 58%. Risk for development of active inflammation (PDAI  $\geq$  7) was significantly higher in patients with preoperative extraintestinal manifestations (OR 2.7, 95%CI 1.1-6.4, p=0.03). Patients who had had ankylosing spondylitis (AS) or iritis were especially at risk. Positive titres of perinuclear antineutrophil cytoplasmic antibodies (pANCA) were associated with inflammation in the proximal ileum; 80% of patients with high pANCA levels (>100) had pouchitis. Current smokers tended to have a more benign

disease course. In patients with pouchitis, faecal concentrations ( $\log_{10}$  CFU/g) of anaerobes and aerobes were significantly higher ( $p= 0.007$ ). Degree of villous atrophy and colonic metaplasia were both associated with faecal bacterial flora. All the patients with conventional ileostomies showed normal mucosal morphology without inflammation. In pouch patients with subtotal or total villous atrophy (32.7%), serum levels of albumin, calcium, total cholesterol, triglycerides, and vitamin E were significantly reduced ( $p < 0.05$ ). The lowest bile acid and vitamin B<sub>12</sub> absorption rates were seen in patients with inflammation in the proximal limb. Vitamin D deficiency occurred in 10.6%, and vitamin A and B<sub>12</sub> deficiency in about 5%. In the lumbar spine, 37% of the pouch subjects with subtotal to total villous atrophy had osteopenia ( $Z$  score  $< -1$ ), whereas none of the IPAA patients with normal villous structure had reduced bone densities in the spine or femoral neck. The highest prevalence of osteopenia (66.7%) was found among those patients with inflammation in the proximal limb of the pouch.

A single-strain probiotic bacterium supplementation *Lactobacillus* GG, did balance the faecal bacterial flora, but, based on clinical or endoscopic response, was ineffective as primary therapy. In terms of overall satisfaction, the patient groups were similar, with 90% of conventional ileostomy and 89% of ileal pouch-anal anastomosis patients satisfied.

**Conclusions:** A strong correlation between AS, iritis, and pouchitis suggests a common link in their pathogenesis. Metabolic consequences after IPAA are associated with pouchitis, grade of villous atrophy, and extent of inflammation in the remaining ileum. Vitamin B12 and D levels should be occasionally checked. Patients with extraintestinal manifestations and high pANCA levels should be counselled on their high risk for developing chronic pouchitis after surgery and on their potential need for long-term antibiotic treatment. Patients with conventional ileostomies preserve better mucosal morphology, have excellent metabolic outcome, and a good quality of life. Patients with chronic pouchitis will need long-term follow-up.

## **INTRODUCTION**

Ileal pouch-anal anastomosis (IPAA) has been the operation of choice following proctocolectomy for ulcerative colitis (UC) and familial adenomatous polyposis over the past 20 years. The most frequently observed long-term complication of IPAA is acute and/or chronic inflammation of the ileal reservoir, called pouchitis. The aetiology of pouchitis is unknown: the role of genetic susceptibility, faecal stasis with bacterial overgrowth, an altered balance in luminal bacteria, nutritional deficiencies, ischaemic complications of surgery, and recurrence of UC have inspired speculation. Its association with extra-intestinal manifestations supports the hypothesis that pouchitis represents ulcerative colitis in the small bowel. All ileal reservoirs show bacterial overgrowth. As a response to an altered intraluminal environment, chronic inflammation with villous atrophy and incomplete colonic metaplasia occur.

There is considerable literature on the technical aspects of surgery, on functional outcome, and on pouchitis incidence. Less attention has been paid to the long-term metabolic consequences that may occur after changes in mucosal morphology and inflammation. This thesis aims to address factors associated with ileal pouch inflammation and its long-term consequences and prognosis.

## REVIEW OF THE LITERATURE

### 1. Ulcerative colitis

Ulcerative colitis is characterised by recurrent episodes of non-infectious inflammation in the mucosal layer of the colon (Edwards and Truelove 1963, 1964). It may present at any age, but occurs more often in the second or third decade of life, with another peak suggested in the 60s (Calkins and Mendeloff 1986). Men and women are equally affected. Annual incidence is around 7 cases per 100 000 population (Probert *et al.* 1992). The inflammation process almost invariably involves the rectum and may extend into the proximal portions of the colon. Confluent inflammation and shallow ulceration extend proximal from the anal margin. Virtually all patients with UC have rectal bleeding or bloody diarrhoea. Other typical symptoms are urgency, tenesmus, lower abdominal cramps, and pain with defecation. In adults at presentation about 55% have proctitis, 30% left-sided colitis (the proximal limit being below the splenic flexure), 15% extensive colitis (involving the transverse colon) or pan colitis. Of patients with proctitis, proximal extension occurs in less than 30% (Meucci *et al.* 2000). Ulcerative colitis is associated with periods of flare-up when the disease becomes more active, and periods of remission or inactivity. A large Danish study has examined a total of 1161 patients with UC for 25 years after diagnosis (Langholz *et al.* 1994). In that study, distribution of disease activity was remarkably constant each year, with approximately 50% of patients in clinical remission. The cumulative probability of relapse was 90% after 25 years of follow-up. The course of the disease changed between remission and relapse without significant predictors (age, sex, extraintestinal manifestations, and initial localisation), except for disease activity in the initial 2 years after diagnosis. In the subsequent 3 to 8 years after diagnosis, 25% of the patients were in remission; 18% had activity every year; 57% had intermittent relapses. Activity in the first 2 years after diagnosis significantly correlated with an increased probability of disease activity in the next 5 years ( $p=0.00001$ ).

## ***1.1. Diagnosis of ulcerative colitis***

Colonoscopy is the initial procedure of choice for most patients with suspected IBD. Colonoscopy with ileoscopy and biopsy can usually differentiate among ulcerative colitis, Crohn's disease, and other disorders that mimic IBD.

Endoscopy in UC typically reveals the following findings: erythema, loss of the vascular pattern, granularity of the mucosa, friability, oedema, and ulcers. Colonic biopsy can serve to confirm diagnosis. The biopsy characteristically reveals distortion of crypts, acute and chronic diffuse inflammatory infiltrates, goblet cell depletion, crypt abscesses and lymphoid aggregates (Jewell 1998).

### ***1.1.1. Differential diagnosis***

It is particularly important at initial presentation to rule out an infectious disease caused by *Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas*, or *Escherichia coli* 0157:H7. Earlier antibiotic therapy may cause *Clostridium difficile* colitis. Most difficult may be to decide whether the diagnosis is ulcerative colitis or Crohn's disease. The clinical manifestations of Crohn's disease are much more variable than those of UC because of transmural involvement and the variability in extent of disease. Three major endoscopic findings specific for the diagnosis of Crohn's disease may help to distinguish it from UC: aphthous ulcers, cobblestoning, and discontinuous lesions (Pera *et al.* 1987). Several years may pass after presentation before clinical evolution permits any firm decision. In Crohn's disease, histologic specimens may show focal inflammation, submucosal involvement, granulomas, goblet-cell preservation, transmural inflammation, and fissuring.

Otherwise, the differential diagnosis includes anal fissure, collagenous colitis, pseudomembranous colitis, ischaemic colitis, diverticulitis, and colonic tumours (Jewell 1998).

## 1.2. Pathogenesis

### 1.2.1. Environmental factors

Data from patients and work in animals regarding intestinal inflammation suggest that UC results from environmental factors triggering a breakdown in the regulatory constraints on mucosal immune responses to enteric bacteria in genetically susceptible individuals (Fiocchi 1998, Sartor 2000). The low rates of concordance in monozygotic twin pairs with UC (6-14%) compared with rates for with Crohn`s disease (44-50%) are the strongest evidence that environmental factors are more important than genetic factors in UC pathogenesis (Orholm *et al.* 2000, Tysk *et al.* 1988).

### 1.2.2. Smoking

Ulcerative colitis affects predominantly non-smokers and ex-smokers (Calkins 1989). This protective effect against ulcerative colitis and detrimental effect on Crohn`s disease remains the most puzzling, but also the most consistent effect (Thomas 2000). Cessation of smoking increases the risk for developing UC above that of never-smokers. This increased risk persists during the 2 to 3 years following smoking cessation (Motley *et al.* 1987). Nicotine is probably the main active ingredient in this association, but the mechanisms remain unknown. Cigarette smoking has effects on cellular (Miller *et al.* 1982) and humoral immunity (Srivastava *et al.* 1991), and raises colonic mucus production (Cope *et al.* 1986). Both smoking and nicotine reduce colonic motility (Coulie *et al.* 2001). Although smoking seems to have no effect on intestinal permeability, in UC, colonic mucosal concentrations of the proinflammatory cytokines interleukin-1 $\beta$  and interleukin-8 are significantly lower in smokers than non-smokers (Sher *et al.* 1999). In-vivo studies have shown that nicotine also has an inhibitory effect on T-helper-2 cell (Th-2) function, which predominates in UC, but has no effect on Th-1 cells (Madretsma *et al.* 1996).

### 1.2.3. Diet

Dietary factors have been considered a possible risk factor for UC. In some studies, IBD patients have been less likely to be breast-fed than controls (Bergstrand *et al.* 1983, Corrao *et al.* 1998). Glassman *et al.* (1990) reported that the frequency of symptoms compatible with cow's milk protein sensitivity during infancy was greater in UC (20.9%) than in a control population (2.8%).

Geerling *et al.* (2000) reported that high intake of mono- and polyunsaturated fat and vitamin B6 may enhance the risk for developing UC. Reif *et al.* (1997) reported that the composition of the preillness diet was related to subsequent development of UC. Sucrose and fat consumption were associated with increased risk for UC, but intakes of fructose, fluid, magnesium, vitamin C, and fibre from fruit were associated with reduced risk.

Although many foods and food components have been suggested to play a role in causing IBD, results are equivocal. However, evidence increases that fermentable dietary fibres and prebiotics can modulate various properties of the immune system, including those of the gut-associated lymphoid tissues (GALT). Changes in intestinal microflora that occur with consumption of prebiotic fibres may potentially mediate immune changes via: direct contact of lactic acid bacteria or bacterial products with immune cells in the intestine, production of short-chain fatty acids from fibre fermentation, or changes in mucin production (Schley and Field 2002).

### 1.2.4. Role of intestinal flora in mucosal inflammation

The colon has the highest bacterial concentrations in the gastrointestinal tract. More than 75% of the wet weight of faecal output is composed of bacterial cells. Each gram of faeces is thought to contain  $1 \times 10^{12}$  microbes, of an estimated 50 genera, belonging to over 400 separate species (Savage 1977, Conway 1995). The development of "spontaneous" colitis in rats and mice appears to require the presence of luminal flora; colitis does not occur in any of several mutant strains when they are maintained in a germ-free environment, but develops rapidly when such mice are colonised by commensal bacteria (Rath *et al.* 2001). This suggests that the luminal flora is a requisite and perhaps central factor in the pathogenesis of UC. Some commensal organisms can

become pathogenic in appropriate circumstances, and the magnitude of this balancing act is illustrated by the similarity between proteins of the harmless commensal *Escherichia coli* and its pathogenic derivatives. Burke and Axon (1988) showed distinct adherent strains of *E. coli* in the colonic mucosa of patients with UC, but this has not been confirmed by others (Walmsley 1998).

Colonic mucus and mucosal barrier abnormalities occur in UC patients (Podolsky and Isselbacher 1984, Rhodes 1989), as well as an increased number of surface-adherent and intra-cellular bacteria in the colonic epithelium with inflammatory bowel disease (IBD) (Schultsz *et al.* 1999, Swidsinski *et al.* 2002).

The high prevalence (about 70%) of pANCA in UC (Saxon *et al.* 1990) and its even higher prevalence in patients with primary sclerosing cholangitis (Duerr *et al.* 1991), and in refractory left-sided UC are the most reproducible data supporting non-epithelial autoimmunity. However, evidence that pANCA is a marker of genetic susceptibility to UC has been less convincing (Papo *et al.* 1996). Rather, it seems to be a marker of underlying immune dysregulation. Recent studies have revealed that expression of this marker antibody reflects an immune response to the antigen products of enteric bacteria (Seibold *et al.* 1998, Cohavy *et al.* 2000). Seibold *et al.* (1998) demonstrated that pANCA cross-reacts with mouse caecal bacterial antigen. In addition, Cohavy *et al.* (2000) discovered that pANCA cross-reacts with *Bacteroides caccae* and *E. coli* commensal bacterial proteins, implicating colonic bacterial proteins as targets of the genetically-determined host immune response in UC that generates pANCA.

#### *1.2.5. Role of the immune system*

The intestine contains gut-associated lymphoid tissue (GALT) which protects it from stimulation by the numerous antigens in the intestinal lumen. When the immunological homeostasis of the GALT is destroyed by exaggerated antigens, or dysregulation of GALT is induced by unknown agents, inflammation of the intestine may occur. Substantial progress has been made in characterising immune-cell populations and inflammatory mediators in patients with inflammatory bowel disease and murine models (Fiocchi 1998). Reasonable consensus exists that the mucosa of patients with established Crohn's disease is dominated by CD4+ lymphocytes with a type 1 helper-T-

cell (Th1) phenotype. The mucosa in patients with UC may be dominated by CD4+ lymphocytes with an atypical type helper-T-cell (Th2) phenotype.

Cytokines play a central role in modulation of the intestinal immune system. They are produced by lymphocytes (especially T-cells of the Th1 and Th2 phenotypes), monocytes, intestinal macrophages, granulocytes, epithelial cells, endothelial cells, and fibroblasts (Aggarwal and Puri. 1994). They have proinflammatory functions interleukin-1, IL-1; tumor necrosis factor, TNF; IL-6, IL-8, IL-12 or antiinflammatory functions (interleukin-1-receptor antagonist, IL-1ra; IL-4, IL-10, IL-11; transforming growth factor $\beta$ , TGF  $\beta$  (Sartor 1994, Elson 1995). In IBD, mucosal and systemic concentrations of many pro- and antiinflammatory cytokines are elevated. Mucosal proinflammatory cytokine production correlates with endoscopic activity of UC (Ishiguro *et al.* 1999). An imbalance occurs in the IL-1/IL-1ra ratio between proinflammatory and antiinflammatory cytokines in the inflamed mucosa of patients with Crohn's disease, UC, diverticulitis, and infectious colitis (Casini-Raggi *et al.* 1995). Furthermore, inhibition of proinflammatory cytokines and the supplementations with antiinflammatory cytokines reduced inflammation in animal models, such as the dextran sulfate colitis model (Sivakumar *et al.* 2002), the trinitrobenzene acid model (Kanai *et al.* 2001), or the genetically engineered model of IL-10 knockout mice (Rachmilewitz *et al.* 2002).

#### 1.2.6. Appendectomy

Appendectomy before onset of UC may show a protective effect against UC (Rutgeers *et al.* 1994, Russel *et al.* 1997, Reif *et al.* 2001). Appendectomised patients seem to have more benign disease course and reduced risk for colectomy (Naganuma *et al.* 2001). A large nationwide study from Sweden showed that such a protective and beneficial effect was limited to appendectomies performed for an inflammatory condition and before the age of 20 years (Andersson *et al.* 2001). Appendectomy after onset of UC does not seem to alter the subsequent course of the disease (Jarnerot *et al.* 2001). The importance of an early appendectomy has been shown also by animal models (Mizoguchi *et al.* 1996).

### 1.2.7. Genetic factors

A ten-fold increased risk for UC appears among first-degree relatives of patients with UC (Orholm *et al.* 1991). Lifetime risk for UC is higher in Jewish than non-Jewish families (Yang *et al.* 1993). Within the major histocompatibility complex (MHC), the human leucocyte antigen HLA genes were the first analysed. An early investigation in a randomly selected European population found in UC patients a significantly higher frequency of HLA-A11 and HLA-A7 (Asquith *et al.* 1974). In Japanese and Jewish patients, HLA DRB1\*1502 is associated with susceptibility to UC and appears more often in patients requiring higher doses of steroids (Futami *et al.* 1995, Toyoda *et al.* 1993). Studies in other ethnic groups first revealed conflicting results. More recently, however, HLADRB1\*0103 and DRB1\*12, associated with UC in white populations (Satsangi *et al.* 1996a, Duerr and Neigut 1995, Roussomoustakaki *et al.* 1997), have been suggested to predict disease extent and severity. Genome-wide scanning studies have shown linkage between UC and regions of chromosomes 3, 7, and 12 (Satsangi *et al.* 1996b).

Interleukin (IL)-1 $\alpha$  and IL- $\beta$  are major proinflammatory cytokines involved early in the inflammatory cascade. The interleukin 1 receptor antagonist (IL-1 ra), the natural inhibitor of these IL-1 agonists, acts by competitively binding to IL-1 receptors without eliciting signal transduction (Arend 1993). All three proteins are coded by genes in the IL-1 gene cluster on the long arm of chromosome 2 (Nicklin *et al.* 1994). Biological observations both in animal models of colitis (Ferretti *et al.* 1994) and in patients with IBD suggest that an imbalance in the biologically important IL-1 ra/IL-1 ratio may contribute in UC to the chronic inflammatory response (Casini-Raggi *et al.* 1995). Such evidence suggests that the IL-1 gene locus is an appropriate candidate region for determinants of both genetic susceptibility and disease phenotype in UC.

### 1.3. Extraintestinal manifestations and complications

#### 1.3.1. Extraintestinal manifestations

Mechanisms responsible for the extraintestinal manifestations of IBD, the most common of which are listed in Table 1, are not clearly understood. The majority of extraintestinal manifestations are related to immunologic mechanisms and associated with the production of various cytokines (Sartor 1994). A process central to the occurrence of extraintestinal manifestations is the development of self-reactive B cells, which are triggered to produce IgG autoantibodies directed against cell surface targets (Naparstek and Plotz 1993). Shared antigens probably play a significant role in the pathogenesis of extraintestinal manifestations. A colon epithelial protein (p40) has been identified with unique crossreactivity to the biliary tract, skin, eyes, and joints (Das *et al.* 1993). The monoclonal antibody developed against p40 binds specifically to colon epithelial cells. The corresponding epitope reactive to this antibody has been detected in biliary epithelium, in keratinocytes, in the nonpigmented ciliary epithelium of the eyes, and in chondrocytes (Das *et al.* 1990, Bhagat *et al.* 1994). This pattern of selective reactivity matches well the established extracolonic complications in UC.

**Table 1. Extraintestinal manifestations of ulcerative colitis**

	Prevalence %	Reference
<b>Related to activity of colitis</b>		
Peripheral arthritis	10-12	de Vlam <i>et al.</i> 2000, Palm <i>et al.</i> 2001
Erythema nodosum	0.9-4	Orchard <i>et al.</i> 1998, Greenstein <i>et al.</i> 1976
Iritis, uveitis	0.5-3.2	Bernstein <i>et al.</i> 2001, Goudet <i>et al.</i> 2001
Thromboembolism	1.3	Talbot <i>et al.</i> 1986
<b>Unrelated to activity of colitis</b>		
Sclerosing cholangitis	2-7	Olsson <i>et al.</i> 1991, Raj <i>et al.</i> 1999
Ankylosing spondylitis,	1.6-7	Monsen <i>et al.</i> 1990, Bernstein <i>et al.</i> 2001,
Sacroileitis	4-18	Dekker-Sayes <i>et al.</i> 1978, de Vlam <i>et al.</i> 2000
Pyoderma gangrenosum	1-2	McCallum <i>et al.</i> 1968, Mir-Madjlessi <i>et al.</i> 1985

### *1.3.2. Metabolic bone disease*

Osteopenia or osteoporosis is a common finding in IBD (Bjarnason *et al.* 1997). Decreased bone mineral density (BMD) has been reported in 31 to 59% of patients with IBD (Abitbol *et al.* 1995, Andreassen *et al.* 1997, Pollak *et al.* 1998). Osteopenia may be more common in patients with Crohn's disease than in those with UC (Ardizzone *et al.* 2000). The mechanism of bone loss in IBD is likely to be multifactorial. Various factors such as extent of small bowel involvement, ileal resection, malnutrition, calcium malabsorption, vitamin-D deficiency, prolonged use of corticosteroids, and reduced physical activity may disturb bone metabolism (Tromm *et al.* 1993, Scharla *et al.* 1994, Abitbol *et al.* 1995, Silvennoinen *et al.* 1995). Strong evidence exists that high inflammatory activity itself, with circulating cytokines, induces bone loss, with suppression of bone formation (Lin *et al.* 1996, Bjarnason *et al.* 1997).

### *1.4. Medical therapy for ulcerative colitis*

Standard medical therapy for UC is predominantly based on the use of oral or topical preparations of aminosalicylates and corticosteroids. Aminosalicylates are used to treat mild-to-moderate disease and are the major drugs for maintaining remission. Corticosteroids and immunosuppressants are used for active disease or refractory disease.

#### *1.4.1. 5-aminosalicylates (5-ASA)*

Sulphasalazine is the oldest (since 1942) and the least expensive of the aminosalicylates, and has been recognised for many years to be useful for preventing flare-ups of UC; 5-aminosalicylates (5-ASA), like mesalazine, the active component of sulphasalazine, has the therapeutic benefits without the majority of the related side-effects. The response rate to 5-ASA administered orally is 54 to 88% (Hanauer 2000). Topical mesalazine has performed better than oral mesalazine and topical corticosteroids for patients with active distal colitis (Sutherland *et al.* 2000), whereas combination oral and topical 5-aminosalicylates has been more effective than either

drug alone for induction (Safdi *et al.* 1997) and maintenance (d'Albasio *et al.* 1997) treatment of mild-to-moderate distal disease. All patients should receive the maximum tolerated dosage of aminosalicylates, including combination therapy if distal disease is present, for at least 4 to 6 weeks before topical or oral corticosteroids are considered.

#### *1.4.2. Corticosteroids*

Corticosteroids are the mainstay of treatment of severe disease. These may be given orally or intravenously, usually in a daily dose of 60 to 80 mg methylprednisolone intravenously, or 40 to 60 mg prednisolone orally. They have a number of potential side effects, which include weight gain and other effects of fluid retention, osteoporosis, increased blood sugar and blood pressure, higher risk of infection, acne, and menstrual problems. After remission is induced, the corticosteroid dose should be tapered over 8 weeks until the drug is discontinued (Hanauer 2000).

#### *1.4.3. Immunosuppressants*

Azathioprine and 6-mercaptopurine can prevent relapse, even when used alone, and in some studies are effective for maintaining remissions in UC that have lasted at least 2 years (Hawthorne *et al.* 1992). The response rate to these immunosuppressants is 50-64% (Hanauer 2000).

Cyclosporin, given intravenously (4 mg/kg) (Lichtiger *et al.* 1994) or orally (4-9 mg/kg) has proven successful in inducing remission in 60 to 80% of patients (Carbonnel *et al.* 1996) in acute relapses of UC. Long-term follow-up studies of patients treated with cyclosporin have shown that 53 to 62% avoid colectomy during a 3- to 5-year follow-up (Stack *et al.* 1998, Cohen *et al.* 1999). Addition of azathioprine or 6-mercaptopurine to treatment regimens is recommended for all patients who respond to cyclosporin (Cohen *et al.* 1999).

## **2. Surgery for ulcerative colitis**

Although drugs and medical therapy are central in the treatment of UC, surgery has an important role in relieving symptoms, addressing serious complications, and improving quality of life.

Indications for surgery in ulcerative colitis are

- failed medical treatment  
chronic disease, recurrent acute exacerbations, steroid dependence  
severe symptoms in an otherwise systemically well patient
- fulminant colitis, perforation, or toxic megacolon
- severe dysplasia or carcinoma of the colonic epithelium

### ***2.1. Choice of operation***

Four operations are available for patients undergoing surgery.

#### ***2.1.1. Conventional proctocolectomy and ileostomy***

Proctocolectomy with construction of a conventional ileostomy has remained the favourite for curative treatment of UC over the years. The evolution of ileostomy technique from spontaneous maturing stoma to eversion stoma according to Brooke (1952), with the advent of enterostomal care (stomatherapy with modern appliances) allows a full, active life for most patients today. Admittedly, some patients do have daily problems, and for others the mere change of body image leads to serious psychosocial consequences (Awad *et al.* 1993).

Panproctocolectomy is a rationale procedure. It removes all diseased mucosa and is in this respect curative. The technique is well established; it can be performed with low mortality and morbidity, and convalescence is rapid. However, complications related to the ileostomy and the perineal wound contribute to considerable postoperative morbidity (Leong *et al.* 1994, Valkamo 1981).

The most common complications of an ileostomy are presented in Table 2.

**Table 2. Long-term complications of conventional ileostomy (Valkamo 1981)**

Complication	Incidence %
Retraction	12.8
Prolapse	2.4
Stenosis	9.6
Peristomal abscess	0.8
Fistula	5.6
Peristomal hernia	8.8

The cumulative risk for needing an ileostomy revision is about 40% over a 10-year period (Carlstedt *et al.* 1987).

For a patient with an ileostomy, an acute gastroenteritis can lead to rapid dehydration and a life-threatening situation. Intravenous fluids and electrolyte replacement are needed until the illness resolves. Patients must be warned of this danger, especially if travelling abroad.

### 2.1.2. Continent ileostomy: Kock pouch

Nils Kock reasoned that a pouch and nipple valve constructed of terminal ileum could store ileal contents internally until emptied voluntarily by passage of a large, soft catheter several times daily, obviating an external appliance. During the 1970s and early 1980s, the Kock continent ileostomy was the primary option for patients requiring surgery for UC but who wished to avoid a permanent conventional ileostomy. Late complications were usually related to the nipple valve. The most common reason for pouch excision was partial or total nipple-valve sliding (Lepistö and Järvinen 2003). An internal fistula sometimes develops through the base of the nipple valve and results in leakage of intestinal contents (Kock *et al.* 1986). Another distressing long-term complication has been pouchitis, which occurs in from 10% to 45% of patients (Church *et al.* 1987, Zuccaro *et al.* 1989).

### 2.1.3. Colectomy with ileorectal anastomosis

Colectomy with ileorectal anastomosis should be considered in patients who have a rectum of adequate distensibility that is not particularly inflamed. The operation is simple, with rapid recovery. However, the disease persists in the rectum. There is a reported failure rate of 10 to 50% due to poor function resulting from persisting inflammation or neoplastic transformation (Nicholls 2002).

### 2.1.4. Ileal pouch-anal anastomosis (IPAA)

The introduction of restorative proctocolectomy for patients with UC in the late 1970s (Park and Nicholls 1978) was greeted with enthusiasm because patients no longer had to accept a permanent ileostomy. Thus, panproctocolectomy with ileal pouch-anal anastomosis has become the treatment of choice for the majority of patients requiring surgery for ulcerative colitis. It permits removal of all pathological large bowel mucosa while preserving intestinal continuity and continence.

The anal sphincter is preserved and an ileoanal anastomosis is constructed after the creation of an ileal reservoir to act as a neo-rectum. The operation may be carried out as a one- or two-stage procedure. There is evidence that long-term outcome is improved if a temporary ileostomy is constructed (Nicholls 2002). Contraindications include Crohn's disease, acute severe colitis, low rectal carcinoma, disseminated carcinoma, and poor anal sphincter. Relative contraindications include the presence of an anal lesion and sclerosing cholangitis (Penna *et al.* 1996). There is now evidence that fertility is reduced by about half in female patients undergoing the operation, and all females of child-bearing age must be fully counselled (Olsen *et al.* 1999).

Creation of a pouch results in stasis, creating a new ileal environment. Because of either stasis or the density of the bacterial population, the mucosa of the pouch develops villous atrophy and crypt elongation and a raised crypt-cell proliferation rate with an infiltration of lymphocytes and eosinophils (Shepherd 1987, de Silva *et al.* 1991a).

After ileal pouch-anal anastomoses, the villous morphology alters as early as in 5 days (Kühbacher *et al.* 1998). Mucin histochemical analysis reveals development of a colonlike mucosal morphology. A switch from sialomucin (produced by the small bowel) to

sulphomucin (produced by the large bowel) occurs in about 50% of patients (Moskowitz *et al.* 1986, Shepherd *et al.* 1987). The histological changes appear to be more prominent in the distal pouch than in the proximal mucosa (Setti Carraro *et al.* 1998). These changes in epithelial cell morphology were initially identified as colonic metaplasia. However, they do not affect the pouch uniformly. Both the adaptive and the inflammatory changes are focal in response to static faecal residue. Other studies, however, suggest that exposure to the faecal stream rather than the stasis appears to be the most important stimulus for observed changes (de Silva *et al.* 1991a, de Silva *et al.* 1991b). The mechanisms underlying colonic metaplasia are likely to be influenced by an interactions between bacteria, short-chain fatty acids (SCFA), and bile acids. Although histological changes resemble those of metaplasia, complete transformation of the epithelium does not occur. Morphological changes in the pouch epithelium may be a prerequisite for the development of pouchitis (Shepherd *et al.* 1993).

### 3. Long-term prognosis after ileal pouch-anal anastomosis

Despite its being a technically challenging procedure, post-operative mortality in IPAA approaches zero (Meagher *et al.* 1998). However, once the pouch is functioning, more than 50% of patients will suffer some of a number of problems (Tables 3 and 4).

**Table 3. Main post-operative complications of ileal pouch-anal anastomosis after UC**

Complication	%	Reference
Pouchitis	30-59	Hurst <i>et al.</i> 1996, Meagher <i>et al.</i> 1998, Simchuk <i>et al.</i> 2000, Tiainen and Matikainen 2000, Heuschen <i>et al.</i> 2001a
Pelvic sepsis	3-5	Meagher <i>et al.</i> 1998, Johnson <i>et al.</i> 2001
Stricture	4-11	Dayton <i>et al.</i> 2002, Prudhomme <i>et al.</i> 2003
Fistulae	1.6-5	Dayton <i>et al.</i> 2002, Breen <i>et al.</i> 1998
Dysplasia/cancer	< 0.5	Thompson-Fawcett <i>et al.</i> 2001, Heuschen <i>et al.</i> 2001b
Pouch failure	0.3-5.3	Keränen <i>et al.</i> 1997, Dayton <i>et al.</i> 2002, Fazio <i>et al.</i> 1995, Lepistö <i>et al.</i> 2002

**Table 4. Long-term functional outcome of ileal pouch-anal anastomosis after surgery for UC**

		Reference
Number of stools/ 24h	5-7	Keränen <i>et al.</i> 1997, Meagher <i>et al.</i> 1998, Johnson <i>et al.</i> 2001
Need for night evacuation	40-80%	Romanos <i>et al.</i> 1997, Keränen <i>et al.</i> 1997
Urgency	6-10%	Romanos <i>et al.</i> 1997
Incontinence		
Occasional	39%	Meagher <i>et al.</i> 1998, Tiainen and Matikainen 2000
Frequent	7%	
Use of retarding drugs	29-75%	Keränen <i>et al.</i> 1997, Romanos <i>et al.</i> 1997, Johnson <i>et al.</i> 2001
Protective pads	17-30%	Meagher <i>et al.</i> 1998, Johnson <i>et al.</i> 2001
Perianal soreness	30-48%	Meagher <i>et al.</i> 1998, Johnson <i>et al.</i> 2001

### **3.1. Pouchitis**

#### *3.1.1. Incidence*

The most frequent long-term complication after ileal pouch-anal anastomosis for UC is pouchitis, a non-specific inflammation of the ileal reservoir (Sandborn 1994, Kühbacher *et al.* 1998), but a standardised definition of and diagnostic procedures for pouchitis are lacking. Reported rates of incidence of pouchitis in patients operated on for UC range between 30 and 59% (Simchuk *et al.* 2000, Hurst *et al.* 1996, Meagher *et al.* 1998, Heuschen *et al.* 2001a). Acute pouchitis has a much higher frequency than chronic pouchitis. Penna *et al.* (1996) reported a cumulative risk for developing pouchitis at one, two, five, and ten years after ileal pouch construction of 16, 23, 36, and 46%, respectively, for patients with UC. Keränen *et al.* (1997) found at 11 years a cumulative overall risk for pouchitis of 28% and a cumulative risk for 5% for chronic pouchitis. Luukkonen *et al.* (1994) demonstrated in UC patients a cumulative risk of developing chronic pouchitis of 5% at 4 years and 7% at 6 years.

A considerable number of cases of pouchitis have proven to be caused by surgical complications (secondary pouchitis) specific to the ileal reservoir and ileoanal anastomosis procedure (pouch-anal fistulas and peripouchal abscesses, outlet obstruction) (Scott and Phillips 1989, Fleshman *et al.* 1988, Fischer *et al.* 1993, Galandiuk *et al.* 1990, Belliveau *et al.* 1999, Heuschen *et al.* 2001a). Differentiation

between primary and secondary pouchitis is important because it determines the mode of therapy. Secondary pouchitis caused by surgical complications requires surgical treatment in nearly all cases (Heuschen *et al.* 2001a).

### 3.1.2. Symptoms

Typical predominant symptoms of acute exacerbation of pouchitis are an increase in stool frequency with loose to watery stool. Abdominal cramping, bleeding per anus and systemic malaise are less common. Exacerbation of extraintestinal manifestations such as arthralgia, arthritis, iritis, erythema nodosum, and pyoderma gangrenosum occur infrequently (Lohmuller *et al.* 1990, Goudet *et al.* 2001).

Shen *et al.* found that symptoms, endoscopic findings, and histological abnormalities do not correlate with each other, which further shows that symptoms alone do not predict the finding of pouch inflammation. In addition, endoscopic and histologic inflammation are not always associated with the presence of severe symptoms, as demonstrated by the fact that 36% of patients with minimal symptoms had significant endoscopic and histologic inflammation. And vice versa, 25% of patients with a high symptom score suggestive of pouchitis did not fulfill criteria for the diagnosis of pouchitis (Shen *et al.* 2001a). Thus, the diagnosis of pouchitis should always be made on the basis of clinical, endoscopic, and histologic features (Moskowitz *et al.* 1986, Madden *et al.* 1990, de Silva *et al.* 1991b, Sandborn *et al.* 1994a, Luukkonen *et al.* 1994, Veress *et al.* 1995, Shen *et al.* 2001a).

### 3.1.3. Endoscopic findings and histologic changes

During endoscopy, the presence of mucosal oedema and erythema, granularity, contact bleeding, friability, loss of vascular pattern, and ulceration indicate pouchitis, but these changes may be patchy (Moskowitz *et al.* 1986, Di Febo *et al.* 1990).

For histological assessment it is important that biopsies are taken from the anterior and posterior wall, avoiding suture lines. Attention should be directed toward acute (polymorph infiltration, crypt abscesses, ulceration) and chronic inflammation (chronic inflammatory cell infiltration, villous atrophy). Other histological findings include low

intra-epithelial lymphocyte density and goblet-cell depletion (Shepherd *et al.* 1987, 1993, de Silva *et al.* 1991b).

#### 3.1.4. Scoring the severity of inflammation

The first disease activity index was developed by Sandborn *et al.* at the Mayo Clinic in Rochester, Minnesota 1994. An overall Pouchitis Disease Activity Index (PDAI) score is calculated from three separate 6-point scales for clinical symptoms, endoscopic findings, and histologic changes. Patients with a total PDAI score of 7 or higher are classified as having pouchitis (Sandborn *et al.* 1994b) (Table 5).

The Heidelberg Pouchitis Activity Score (PAS), presented in the year 2001 by Heuschen *et al.* takes into account also chronic inflammatory infiltration when scoring the severity of pouchitis (Heuschen *at al.* 2001a). In that scoring system, grade I inflammation (score 4-12) is defined as mild adaptive inflammation, because it is almost universal. Grade II inflammation (score 13-24) represents moderate pouchitis. Grade III inflammation (score 25-36) is defined as severe pouchitis (Table 6).

**Table 5. Pouchitis disease activity index (PDAI): maximum 18 points**

Criteria	Score	
Clinical	Post-operative stool frequency	
	Usual	0
	1-2 stools/day more than usual	1
	3 or more stools than usual	2
	Rectal bleeding	
	None or rare	0
	Present daily	1
	Faecal urgency/ abdominal cramps	
	None	0
	Occasional	1
	Usual	2
	Fever	
	Absent	0
Present	1	
Endoscopic	Oedema	1
	Granularity	1
	Friability	1
	Loss of vascular pattern	1
	Mucus exudate	1
	Ulceration	1
Histological	Polymorph infiltration	
	Mild	1
	Moderate + crypt abscess	2
	Severe + crypt abscess	3
	Ulceration	
	<25%	1
≥25%, <50%	2	
>50%	3	

Pouchitis is defined as a total score of  $\geq 7$

Sandborn *et al.* Mayo Clinics Proceedings 1994;64:409-415 (b)

**Table 6. The Heidelberg Pouchitis Activity Score (PAS): maximum 36 points**

CLINIC	Score		Score
Stool frequency/24 hours		Rectal bleeding	
< 8	0	Absent	0
8-10	2	present	3
11-13	4		
>13	6		
Faecal urgency			
absent	0		
present	3		
			Max. 12
<b>ENDOSCOPY</b>			
Oedema		Granularity	
absent	0	absent	0
present	1	present	1
Friability		Erythema	
absent	0	absent	0
mild	1	mild	2
severe	2	severe	3
Flattening of mucosal surface		Ulcerations/Erosions	
absent	0	absent	0
present	2	mild	2
		severe	3
			Max. 12
<b>HISTOLOGY</b>			
Acute histologic inflammation		Chronic histologic inflammation	
Polymorphonuclear leukocyte infiltration		Mononuclear leukocyte infiltration	
absent	0	absent	0
discrete or patchy	1	mild and patchy	1
moderate with (±) crypt abscesses or cryptitis	2	moderate	2
extensive with (±) crypt abscesses or cryptitis	3	extensive	3
Ulceration/Erosions		Villous atrophy	
absent	0	absent	0
mild and superficial	1	minimal	1
moderate	2	partial	2
extensive	3	subtotal/total	3
			Max. 12

Heuschen *et al.* Dis Colon Rectum 2001;44:487-499

### 3.2. Pathogenesis of pouchitis

#### 3.2.1. Bacterial overgrowth

In the pathogenesis of pouchitis, a major contributing factor is faecal stasis with bacterial overgrowth. Most episodes of pouchitis are single attacks usually responding to metronidazole or to antimicrobial combination therapy (Mimura *et al.* 2002). In ileal reservoirs without signs of pouchitis, the microflora closely resembles the flora of the large bowel. This mainly due to the large numbers of anaerobes, resulting in a greater ratio of anaerobes to aerobes (Luukkonen *et al.* 1988, Go *et al.* 1988, Nasmyth *et al.* 1989, Santavirta *et al.* 1991a). The pouches of patients with pouchitis, however, harbour increased concentrations of anaerobes (Onderdonk *et al.* 1992) and aerobes, a reduced ratio of anaerobes to aerobes, with reduced counts of *Lactobacillus* and *Bifidobacterium* (Ruseler-van Emden *et al.* 1994).

These increased numbers of bacteria appear responsible for the increased crypt cell production rate and villous atrophy observed in the pouch mucosa soon after construction of a reservoir (Philipson *et al.* 1975). Nasmyth *et al.* (1989) found a significant correlation between number of isolated *Bacteroides* and the grade of villous atrophy. The greater the number of *Bacteroides* the more severe was the villous atrophy.

#### 3.2.2. Recurrence of ulcerative colitis?

Pouchitis occurs almost exclusively in patients who undergo colectomy for ulcerative colitis. In a recent study, the cases of pouchitis in familial adenomatous polyposis (FAP) patients were all secondary forms (pouch-anal fistula) (Heuschen *et al.* 2001a).

Cytokine production in pouchitis resembles that in ulcerative colitis. Relative to inflamed pouches or to normal ileal mucosa, mucosal biopsy specimens from inflamed pouches have demonstrated an increased CD4:CD 8 ratio, increased expression of T-cell activation markers such as CD25, and an increased number of interferon  $\gamma$ -producing cells (Stallmach *et al.* 1998). In addition, studies have demonstrated an increased expression of proinflammatory cytokines such as interleukin 1 (IL-1), IL-6, IL-8, and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) in ileal reservoirs of patients relative to those in non-inflamed pouches (Gionchetti *et al.* 1994, Patel *et al.* 1995, Ulisse *et al.* 2001).

Neuromediators, including substance P (Keränen *et al.* 1996, Watanabe *et al.* 1998), may thus be able to modify intestinal inflammatory reactions (Anton and Shanahan 1998) by direct regulation of immune cells.

That overproduction of nitric oxide (NO) by inducible NO synthase (iNOS) may contribute to the pathophysiology of both UC and pouchitis (Southey *et al.* 1997) has been suggested in animal models (Southey *et al.* 1997) and in studies of UC and pouchitis (Kimura *et al.* 1998, Rachmilewitz *et al.* 1998). It has been proposed that NO is the main inductor of IL-8 and is associated with an increase in cyclo-oxygenase 2, tumor-necrosis factor (TNF)  $\alpha$ , and IL-1 $\beta$  expression. Because NO has also been shown to be a neurotransmitter in the noradrenergic-noncholinergic inhibitory nerves in the human gut, it is therefore regarded as an important contributor to the motility problems seen in acute UC. Motility studies have shown NO to act more strongly in the UC colon than in the normal colon (Tomita and Tanjoh 1998). Producers of NO are most likely macrophages and neutrophils within the colonic or pouch mucosa (Ikeda *et al.* 1997).

### 3.2.3. Short-chain fatty acids

Stools of patients with active pouchitis have been reported to contain decreased concentrations SCFAs (Sagar *et al.* 1995, Clausen *et al.* 1992). In contrast to these studies, Sandborn and colleagues (1995) could not demonstrate any decreased faecal concentration of short-chain fatty or bile acids. Short-chain fatty acids are an important energy source for the colonic epithelium, and their deficiency may cause diversion colitis (Harig *et al.* 1989). Butyrate is a major fuel for the colonic mucosa and may inhibit cell proliferation and stimulate cell differentiation (Phillips *et al.* 1995). It has been proposed that in the pouch, low pouch concentrations of fermentable saccharides reduce the production of short-chain fatty acids by faecal bacteria (Clausen *et al.* 1992). Nasmyth's group (1989) observed a negative correlation between concentration of butyrate in the pouch effluent and severity of villous atrophy of the pouch mucosa. Pouchitis may be due to a reduction in substrate availability, leading in turn to low SCFA, and it may respond to instillation of SCFA into the pouch by enema, but the benefit varies depending on the report (de Silva *et al.* 1989, Wischmeyer *et al.* 1993, Ambroze *et al.* 1993).

#### 3.2.4. Mucosal ischaemia

Doppler studies have been shown that mucosal blood flow in pelvic reservoirs is significantly lower than mucosal bloodflow in conventional ileostomies (Perbeck *et al.* 1985). Transient mucosal ischaemia may cause oxygen-derived free radical production by xanthine oxidase inhibitor, in patients with acute and chronic pouchitis. Allopurinol is a scavenger of oxygen-derived free radicals. To investigate the role of this xanthine oxydase inhibitor, Joelsson *et al.* (2001) conducted a study in 184 patients with ileal pouch-anal anastomosis randomized to receive postoperative prophylactic allopurinol 100 mg twice daily or placebo. The allopurinol failed to reduce risk for a first attack of pouchitis.

Moreover, the same surgery is used to treat patients with familial adenomatous polyposis (FAP), in whom the incidence of pouchitis is very low (Kartheuser *et al.* 1996), which suggests that ischaemia is not the cause of pouchitis (Mahadevan and Sandborn 2003).

#### 3.3. Risk factors for the development of pouchitis

Several independent risk factors for pouchitis have been identified.

- Previous course of the disease (high relapse rate, chronic refractory disease)
- Presence of extraintestinal manifestations such as primary sclerosing cholangitis
- High pANCA (> 100 ELISA units /ml)
- Cessation of smoking
- Genetic factors

Episodes of pouchitis are associated with extra-intestinal manifestations characteristic of UC. Furthermore, an association exists between such manifestations occurring prior to surgery and subsequent development of pouchitis. Patients with primary sclerosing cholangitis (PSC) are particularly at risk for pouchitis (Penna *et al.* 1996, Aitola *et al.* 1998). The cumulative risk for pouchitis at 10 years after ileal pouch-anal anastomosis has been 45.5% without PSC and 79% for patients with PSC (Penna *et al.* 1996).

Orthotopic liver transplantation in patients with sclerosing cholangitis and ileal pouch anal anastomosis does not alter risk for developing pouchitis (Zins *et al.* 1995).

The presence of antibodies directed against neutrophil granulocytes and which show a characteristic perinuclear binding pattern (pANCA) was initially regarded as a strong risk factor and has been advocated to play a primary role in pouchitis pathogenesis. Some studies have shown that proctocolectomy leads to a reduction in pANCA titres (Esteve *et al.* 1996, Aitola *et al.* 1995). However, a recent study by Fleshner *et al.* (2001) reported that high levels of pANCA (>100 ELISA units/ml) before colectomy are significantly associated with development of chronic pouchitis after IPAA. The cumulative risk for chronic pouchitis in these high-pANCA patients was 56%, compared to 22% in medium-level (40-100 EU/ml), 16% in low-level (<40 EU/ml), and 20% in pANCA-negative patients (p=0.005) (Fleshner *et al.* 2001).

Smokers seem to have fewer episodes of pouchitis than do nonsmokers or former smokers. Pouchitis occurred in 18 of 72 non-smokers, in 4 of 12 ex-smokers but in only one of 17 smokers (Merret *et al.* 1996). It appears that the effect of smoking on pouchitis parallels that seen in UC (Thomas *et al.* 1998).

Carter and colleagues (2001) reported that after IPAA for UC, the interleukin 1 receptor-antagonist gene allele 2 predicts pouchitis. Patients with pouchitis had a higher allele-2 carriage rate than did those without pouchitis (72% vs. 45%), and Kaplan-Meier survival analysis showed that allele-2 carriers had a significantly higher incidence of pouchitis than did noncarriers (log-rank test, 6.5).

### **3.4. Consequences of colectomy and IPAA**

#### **3.4.1. Anaemia**

In a recent study by Tiainen and Matikainen (2000) the prevalence of anemia in IPAA cases at the time of the study visit was 20.8%. Severe or chronic pouchitis exposed patients to anemia, with iron deficiency occurring in 10.4%.

### 3.4.2. Electrolyte imbalance

Changes in water and sodium balance after IPAA are similar to those after conventional ileostomy. Santavirta *et al.* (1991b) studied water and electrolyte balance in 30 patients with IPAA, 10 patients with conventional ileostomy, and 9 non-operated patients with quiescent ulcerative colitis. Serum chloride in IPAA patients was significantly lower than in the other groups. Daily urinary loss of sodium in non-surgical patients was significantly higher than in patients with an ileal pouch or conventional ileostomy. Daily faecal weight, urinary volume, and urinary excretion of sodium were similar in patients with IPAA and ileostomy. Using tritiated water and a bromide dilution technique, Christie *et al.* (1990) showed that in patients with IPAA the body content of water and extracellular fluid are normal.

### 3.4.3. Malabsorption

Resection of the ileum together with bacterial colonisation may lead to villous atrophy and thus to loss of absorptive mucosa. These changes may lead to reduced absorption of bile acids, lipids, and vitamin B<sub>12</sub>.

Increased faecal bile acid excretion after IPAA has been well documented (Hylander *et al.* 1991, Natori *et al.* 1992). Santavirta *et al.* (1990) found that IPAA patients with low retention of bile acids had more severe villous atrophy than those with high retention. However, bile acid malabsorption is seldom severe enough to impair micelle formation and cause steatorrhea. Among patients 3 months after ileostomy closure, Hylander *et al.* (1991) found moderate steatorrhea in approximately 30%, but faecal fat excretion normalised with time. Decreased cholesterol absorption, with lower serum total and LDL cholesterol and LDL triglycerides has been reported after IPAA (Hakala *et al.* 1997a, Hakala *et al.* 1997b), as have fat-soluble vitamin deficiencies in patients with inflammatory bowel disease (Fernandez-Banares *et al.* 1989, Geerling *et al.* 1999). Reduced vitamin B<sub>12</sub> absorption (Schilling test) has been shown in 10 to 30% of patients with IPAA, and clinically significant vitamin B<sub>12</sub> deficiency has been documented in 3 to 9% of such patients (Bayat *et al.* 1994, M'Koma *et al.* 1994a, M'Koma *et al.* 1994b).

#### 3.4.4. Irritable pouch syndrome

A substantial number of symptomatic patients after IPAA do not meet the criteria for either pouchitis or cuffitis (endoscopic and histological inflammation of the rectal cuff). These patients can be classified as having irritable pouch syndrome (IPS). There is an overlap of symptoms among patients with pouchitis, cuffitis, and IPS. Increased stool frequency, urgency, and abdominal cramps are the most common symptoms. A 2002 study by Shen *et al.* reported that 46.2% of patients with IPS responded to antidiarrheal, anticholinergic, and/or antidepressant therapies. An endoscopic evaluation can differentiate among these groups.

### 3.5. Therapy for pouchitis

#### 3.5.1. Medical therapy

Medical treatment for pouchitis is largely empirical. The hypothesis that faecal stasis and bacterial overgrowth may be of importance in the pathogenesis of pouchitis led clinicians to treat patients with antibiotics, and these have become the mainstay of treatment, in the absence of controlled trials.

Most patients with acute pouchitis respond quickly to metronidazole of 1 to 1.5 g/day (Hurst *et al.* 1996, Sagar and Pemberton 1997). This antibiotic reduces leukocyte infiltration in the mucosa of the pouch (Kmiot *et al.* 1993) and faecal bacterial counts of *Bacteroides*. Madden *et al.* (1994) carried out a double-blind, randomised, placebo-controlled, crossover trial to assess the efficacy of 1.2 g/day of metronidazole orally in 11 patients with chronic unremitting pouchitis. Metronidazole was significantly more effective than placebo in reducing stool frequency (73% vs. 9%), though without improvement in endoscopic appearance and histologic grade of activity. However, 55% of metronidazole-treated patients experienced side-effects including nausea, vomiting, abdominal discomfort, headache, skin rash, and metallic taste.

Nygaard and colleagues (1994) administered topical metronidazole (40-160mg/day), which induced clinical improvement within a few days without side-effects and with a decrease in concentrations of anaerobic bacteria.

Shen and colleagues (2001b) treated seven patients with ciprofloxacin at 1 g/day and nine patients with metronidazole 20 mg/kg/day for a period of 2 weeks. This study showed that both antibiotics were effective and reduced the total PDAI scores and led to a significant improvement in symptoms and endoscopic and histologic scores. However, ciprofloxacin led to a greater degree of improvement and was better tolerated.

Recently, budesonide corticosteroid enemas have been shown to be equally effective but better tolerated than oral metronidazole in a double-blind controlled trial (Sambuelli *et al.* 2000). While no data have been published on the efficacy of oral 5-ASA, uncontrolled studies have suggested that 5-ASA either as suppositories or enemas in treatment of acute pouchitis may help (Miglioli *et al.* 1992).

Treatment of chronic refractory pouchitis, which includes patients who fail to respond to antibiotics and those who continuously relapse once antibiotic treatment ceases, has been difficult and disappointing. Among these patients misdiagnosis of Crohn's disease should be excluded. Approximately 5% of IPAA procedures are performed in patients whose primary diagnosis of UC is revised at some point after surgery to Crohn's disease. In these cases, therapeutic treatment is the same as for pelvic and perianal Crohn's disease.

Gionchetti and colleagues (1999) carried out a combined antibiotic treatment in chronic treatment-resistant pouchitis. Treating 18 patients who did not respond to a standard 4-week treatment (metronidazole or ciprofloxacin or amoxicillin/clavulanic acid) orally with rifaximin 2 g/day and ciprofloxacin 1 g/day for 15 days. Of these 18 patients, 16 (88.8%) either improved or went into remission; the median PDAI decreased significantly from 11 to 4. A significant decrease occurred in total anaerobes and aerobes, enterococci, lactobacilli, bifidobacteria, and bacteroides in faecal samples.

Mimura and colleagues (2002) treated 44 patients with active or refractory pouchitis with ciprofloxacin 500 mg b.i.d and metronidazole 500 mg b.i.d for 4 weeks; 82% went into remission, with no serious side-effects noted.

### 3.5.2. Probiotics

Because of the key role of the intestinal microflora in the development of IBD, manipulating this component provides a very intriguing novel approach. Probiotic

bacteria are nonpathogenic microorganisms belonging to the normal gastrointestinal flora that confer a health benefit by altering the indigenous microflora (Bengmark 1998, Fuller 1989). Lactobacilli, bifidobacteria, and other members of the resident microflora are commonly selected as probiotics. They can influence intestinal physiology either directly or indirectly through modulation of the endogenous ecosystem or immune system (Marteau *et al.* 2001) Preliminary trials of probiotics in maintenance treatment of chronic pouchitis are encouraging (Gionchetti *et al.* 2000a).

Gionchetti *et al.* (2000a) demonstrated the effectiveness of the combined probiotic preparation VSL#3<sup>™</sup> (Yovis:Sigma-Tau, Pomezia, Italy) in preventing flare-ups of chronic pouchitis. This product contains 300 billion cells/g of viable lyophilized bacteria of four strains of lactobacilli (*L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* spp. *bulgaricus*), three strains of bifidobacteria (*B. longum*, *B. breve*, *B. infantis*) and one strain of *Streptococcus salivarius* spp. *thermophilus*. In that study, 40 patients who had achieved clinical and endoscopic remission after one month of combined antibiotic treatment (rifaximin 2 g/day + ciprofloxacin 1 g/day) were randomised to receive either VSL#3<sup>™</sup> 6 g/day or an identical-appearing placebo for 9 months. All patients who received placebo relapsed (an increase of at least 2 points in the clinical portion of the PDAI scoring system). In contrast, 17 of the 20 patients treated with VSL#3<sup>™</sup> were still in remission at the end of the study. More recently, the same probiotic product was also shown to be significantly superior to placebo in the prevention of pouchitis onset within the first year after surgery (Gionchetti *et al.* 2000b).

## AIMS OF THE STUDY

The aims of the present study were to analyse:

1. factors associated with changes in mucosal morphology after ileal pouch-anal anastomosis
2. factors associated with ileal inflammatory activity: previous course of ulcerative colitis, extraintestinal manifestations, pANCA level, and smoking habits
3. metabolic consequences in patients with IPAA compared with patients with conventional ileostomies, evaluating whether those with IPAA need long-term follow-up
4. whether modification of ileal microbial flora by a single strain probiotic bacteria, *Lactobacillus rhamnosus* GG, can ameliorate inflammation process in patients with previous pouchitis history
5. long-term prognosis, and to compare life satisfaction in patients with conventional ileostomy with that of patients with ileal pouch-anal anastomosis.

## **PATIENTS AND METHODS**

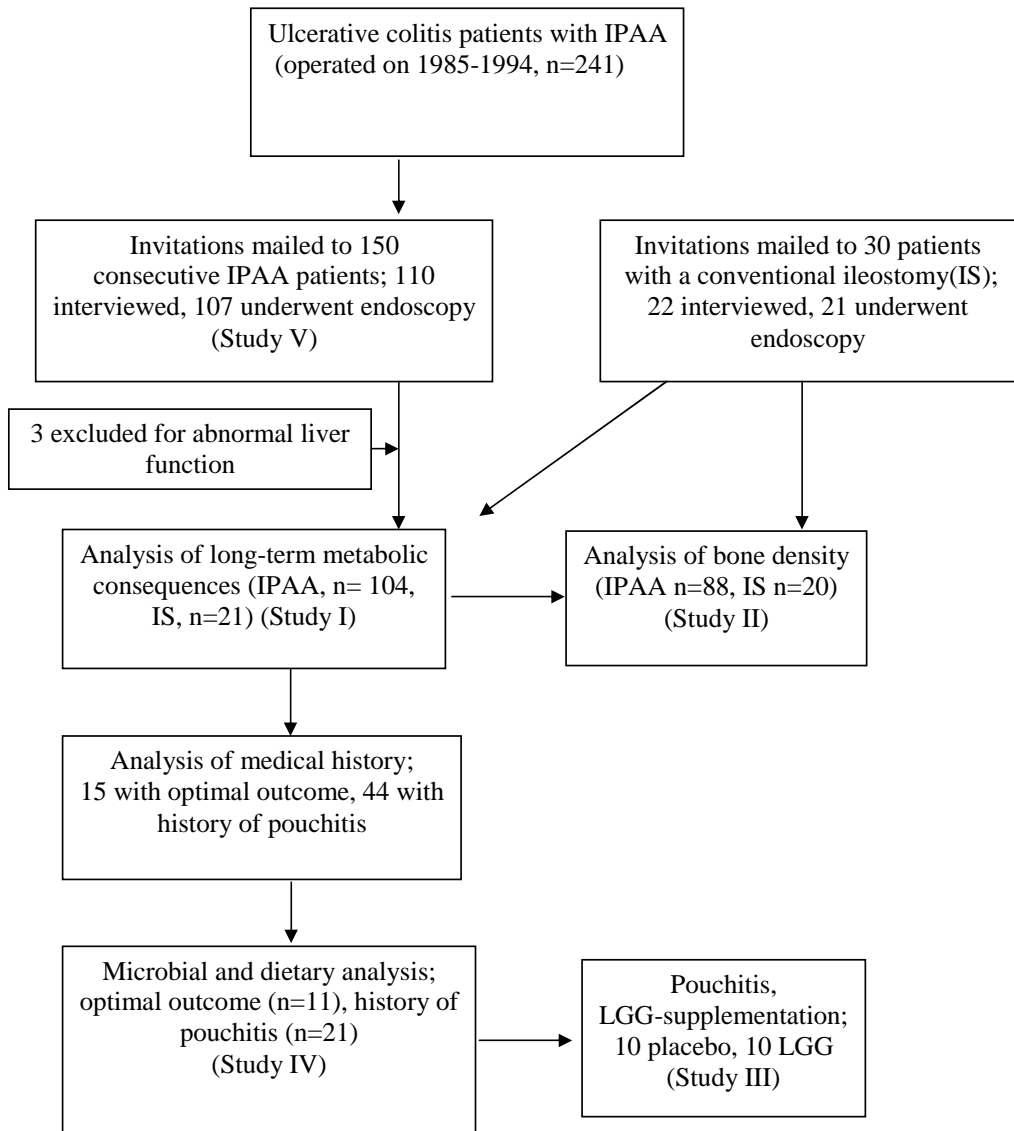
### **1. Patients**

Study subjects were recruited from ileostomy- and IPAA-patient registers from Helsinki University Central Hospital. The study was carried out at the Department of Gastroenterology in the Helsinki University Central Hospital between January 1997 and December 2000.

The original study population comprised a total 128 study subjects: 107 with ileal pouch-anal anastomosis and 21 ileostomy controls. Subjects eligible for study participation were those (n= 241), who had undergone restorative proctocolectomy with an ileal pouch-anal anastomosis (IPAA) for ulcerative colitis between 1985 and 1994 at the Department of Surgery of the Helsinki University Central Hospital. The standard operation was a procedure using the two-limbed J-shaped ileal pouch with or without covering ileostomy. Invitations were mailed to the 150 consecutive patients living in the main catchment area of our hospital, and 110 of them expressed an interest in participating. Subjects operated on earlier at the same unit for ulcerative colitis with a conventional ileostomy (n= 30), were asked to participate as controls. The diagnosis of ulcerative colitis was established in all subjects by standard endoscopic, histological, radiological, and clinical assessment, and by proctocolectomy specimens. The clinical characteristics of the study population are presented in Table 7.

This study complies with the Helsinki Declaration, and the study protocol was accepted by the Ethics Committee of Helsinki University Central Hospital.

**Figure 1. Flowchart of patients with IPAA and conventional ileostomy**



**Table 7. Clinical characteristics of study population**

	IPAA	Ileostomy
Total n	107	21
Sex, M:F	54:53	9:12
Mean age, yr (range)	45.1 (23 - 69)	51.7 (37 - 68)
Follow-up, yrs (range)	7.5 (5 - 12)	19.2 (16 - 28)
BMI, kg/m <sup>2</sup>	25.2 ± 1.0	26.3 ± 0.9
Duration of UC, yrs	6.9 ± 0.6	4.0 ± 1.3
Extent of UC, pancolitis: left-sided	82:25	20:1
Indication for surgery, fulminant:chronic	21:73	4:17
Dysplasia (%)	13 (12)	0

Values presented as number of patients or as means ± SEM

### ***1.1. Study protocol***

Study subjects were examined at the out-patient clinic of the Department of Gastroenterology at Helsinki University Central Hospital.

A flowchart of the study subjects during the studies is presented in Figure 1.

During their first visit, a physical examination was done, and clinical symptoms (stool frequency, rectal bleeding, urgency, fever) and medication were recorded. Patients` histories were collected: onset and duration of ulcerative colitis (in years); site and extent of disease (pancolitis vs. left-sided involvement); indication for IPAA (fulminant, chronic active, dysplasia); operation type (one-stage vs. two-stage); and follow-up time after IPAA (in years). All subjects completed a questionnaire concerning their overall symptoms including smoking habits and satisfaction with life.

## **2. Methods**

### ***2.1. Endoscopic and histological examination***

A pouch endoscopy was performed with a flexible endoscope, and findings (edema, granularity, friability, loss of vascular pattern, mucous exudate, ulceration) scored from 0 to 1 each, according to Sandborn *et al.* (1994b). During the examination of the ileal pouch or ileostomy, mucosal biopsies were obtained at four separate sites in the pouches and proximal ileum or 10 cm from the orifice of ileostomies. Biopsies were taken from any

regions exhibiting active inflammation endoscopically or from normal-appearing mucosa in the absence of endoscopic inflammation, avoiding staples and suture lines.

All biopsy specimens were reviewed in a blinded fashion by one pathologist (AK). Serial sections of formalin-fixed paraffin-embedded tissue were stained with haematoxylin and eosin (H&E) and high iron diamin-alcian blue (HID-AB). The H&E-stained sections were scored on a scale of 0 (absent) to 3 (severe) for degree of acute inflammation (polymorphonuclear leukocyte infiltration, ulceration) and for degree of villous atrophy by criteria of Moskowitz *et al.* (1986). The HID-AB stained sections were scored for mucin (0=sialomucin only, 1=predominant sialomucin, 2=predominant sulphomucin, 3=sulphomucin only). Scores were based on the most severe changes evident. The pouchitis disease activity index, PDAI (Clinical symptoms, endoscopic, and acute histologic inflammation) as described by Sandborn served to quantify pouchitis disease activity. Pouchitis was defined as a PDAI  $\geq 7$  (Sandborn *et al.* 1994b). On the basis of villous atrophy, the patients with IPAA were divided into three subgroups: no villous atrophy or partial atrophy as two groups; but those with subtotal or total villous atrophy were considered together as a single group, because of the patchy distribution of inflammation and villous atrophy in the pouch (Shepherd *et al.* 1987).

## **2.2. Biochemical assessment**

After an overnight fast, immediately after pouch endoscopy all patients had blood samples taken for a complete blood count, biochemistry profile, and serum lipid and vitamin measurements. Serum concentrations of albumin, calcium, alkaline phosphatase, and alanine transferase were measured by standard laboratory methods. Serum parathyroid hormone (PTH) (normal 10-60 pg/ml) levels were measured by a commercially available two-site immunoradiometric assay (Intact PTH; Nichols Institute Diagnostics).

Serum total cholesterol and triglycerides were determined by routine hospital methods (kits from Boehringer Diagnostica, Germany).

Vitamin B<sub>12</sub> (normal 170-670 pmol/l) and folate (normal 4.4-18.0 nmol/l) were analysed by competitive protein binding assay (SimulTRACK radioassay kit by ICN Pharmaceuticals, NY, USA)

Vitamin A (normal 1.0-2.8  $\mu\text{mol/l}$ ) and E (normal 12-40  $\mu\text{mol/l}$ ) were determined by high-pressure liquid chromatography (HPLC), and 25-hydroxyvitamin D<sub>3</sub> (normal 20-100 nmol/l) was determined by radioimmunoassay (DiaSorin Inc., Stillwater, MN, USA).

A marker of bone formation, serum osteocalcin (normal range, age-dependent: female 3.8-30, male 11-32  $\mu\text{g/l}$ ) was measured with a human-specific two-site immunoradiometric assay (IRMA; ELSA-OSTEO, Cis Biointernational, France). A marker of bone resorption, the urinary N-telopeptide cross-linked collagen-type 1/creatinine ratio (U-NTX; normal range: female 5-65 nmol/mmol creatinine, male 5-85 nmol/mmol creatinine) was measured by enzyme-linked immunosorbent assay (EIA; Ostex International Inc, Seattle, WA, USA).

Determination of perinuclear antineutrophil cytoplasmic antibodies (pANCA) was performed by conventional indirect immunofluorescence using ethanol-fixed neutrophil slides (Inova Diagnostics, San Diego, CA, USA). A pANCA titre  $\geq 1 : 10$  was considered positive.

### **2.3. Absorption studies**

Absorption of vitamin B<sub>12</sub> with intrinsic factor was measured by the Dicopac test routinely used at Helsinki University Central Hospital (Bayly *et al.* 1971).

Absorption of bile acids was studied by use of the selenenim-labeled synthetic bile salt <sup>75</sup>SeHCAT (taurine conjugate of 23- [<sup>75</sup>Se]-25-homocholic acid). Ten microcuries of <sup>75</sup>SeHCAT in capsule form was given orally to each patient. Selenium radioactivity was measured by whole-body counting with a gamma-camera immediately after ingestion and at 3, 24, and 72 hours. Results were expressed as retention of bile acids (%) at three days (72 hours), SeHCAT/72h% (normal  $\geq 30\%$ ).

### **2.4. Bone mineral density measurements**

Bone mineral density (BMD) ( $\text{g/cm}^2$ ) was measured by dual energy x-ray absorptiometry (DEXA; Hologic QDR 1000, Waltham, MA, USA) at the lumbar spine (L1-L4, posteroanterior view) and left femoral neck levels by standard protocols. In the present study, results of BMD are expressed as the number of standard deviations from

normal values corrected for age and sex (Z score) to better demonstrate any potential disease-specific changes after proctocolectomy. Reference values for men and women were supplied by Hologic. A Z score < -1 was defined as osteopenia.

### ***2.5. Faecal bile acids***

Faecal bile acids were determined from a single stool sample by gas-liquid chromatography (Grundy *et al.* 1965), (HP 5890 series II, Hewlett-Packard, Wilmington, DE, USA) on a capillary column (HP-1, Crosslinked Methyl Siloxane, length 50 m, diameter 0.32 mm, Hewlett-Packard). Results are expressed as the composition of individual bile acids expressed as percentage of total bile acids and µg bile acids/g faeces.

### ***2.6. 7-day food diary***

Participants recorded their habitual diet for 7 days. They were instructed to record everything they ate or drank, using household measures and standard packing units. No vitamin or mineral supplement usage was included in diet records. Nutrient and energy calculations were based on the Finnish Nutrient Data Bank supplied by the Social Insurance Institution Research and Development Centre (Turku, Finland), and carried out with use of software for Windows developed at the Research and Development Centre. Nutrient values have been derived from analyses of Finnish foods and from international food-composition tables.

### ***2.7. Specimens for bacteriologic studies***

Fresh faecal samples were collected prior to endoscopy. The pouch was emptied with water enemas. During flexible pouch endoscopy before biopsy-taking, the mucosa was carefully rinsed with sterile water under direct visual control to remove all possible faecal contamination. We obtained five separate tissue biopsy specimens (three for bacterial culture and two for histology) from the pouch and five from the afferent limb above the pouch. These specimens were taken from the regions exhibiting active inflammation endoscopically. Staples and suture lines were avoided. Tissue biopsy

samples were thoroughly rinsed with downward jets of sterile saline and placed in a Stuart transport tube.

## **2.8. Analysis of bacteriologic specimens**

Bacteriologic samples were immediately transported to the laboratory and processed within 2 hours. Specimens were weighed and ground in thioglycolate medium (1:10) in a tissue grinder. Faecal samples were homogenised, pH measured (Benchtop 420 pH Meter, Orion, USA) and serially diluted ( $10^{-1}$ – $10^{-7}$ ) in prereduced peptone-yeast extract broth (pH 7.0). Appropriate dilutions and aliquots of the homogenates were plated onto several selective and non-selective agar media for the isolation of aerobic and anaerobic microbes. MRS (deMan Rogosa and Sharpe, Merck) agar was used for the isolation of lactobacilli, Brucella agar supplemented with haemin and vitamin K<sub>1</sub> for total counts of anaerobic bacteria, and blood agar (5% sheep blood) for total counts of aerobic bacteria. MRS and Brucella plates were incubated in anaerobic jars filled with a gas mixture (80% N<sub>2</sub>, 10% CO<sub>2</sub> 10% H<sub>2</sub>) and blood plates in 7% CO<sub>2</sub> atmosphere both at 37°C and incubated for up to 14 days. Total counts of the main groups of aerobic and anaerobic bacteria and yeast were enumerated. Different colony morphotypes were recorded, enumerated, and isolated for further study. Isolates were identified by established methods including aerotolerance testing, gram staining, biochemical tests, and enzyme profiling (Summanen *et al.* 1993, Jousimies-Somer *et al.* 1999). The species and clonal identity of selected *Lactobacillus* GG isolates were confirmed by an arbitrarily primed-PCR (AP-PCR) method (Tynkkynen *et al.* 1999).

## **2.9. *Lactobacillus rhamnosus* GG supplementation**

Subjects were randomised either to treatment with gelatin capsules (Gefilus<sup>®</sup>, Valio Ltd, Helsinki, Finland) containing *Lactobacillus rhamnosus* GG,  $0.5-1 \times 10^{10}$  cfu/capsule, with microcrystalline cellulose (MCC) as a filling material, or to MCC only, as a placebo. Each subject received four capsules of *Lactobacillus* GG per day as two equal doses for 3 months. Subjects were asked not to eat probiotic dairy products during the supplementation period.

### ***2.10. Questionnaire***

All subjects completed a questionnaire containing practical questions dealing with patients` functional outcome (i.e., stool frequency/24 h, need for night evacuation, use of retarding drugs, need for protective pads, soiling, perianal or peristomal soreness, and urgency after surgery). Patient satisfaction was evaluated by questions as to overall satisfaction (score 1-5), intestinal symptoms (1-4), any restrictions on diet (open answer), and social activity, such as changes in social interaction (1-2), occupation (1-2), or daily activities (1-2). Sexual function: impotence (1-2), retrograde ejaculation (1-2), dyspareunia (1-2), and smoking and drinking habits were also recorded.

### **3. Statistical Analysis**

All data are expressed as mean  $\pm$  SEM. Comparisons among the patient groups were made by analysis of variance (ANOVA) with the Newman-Keuls test. When variances were unequal or distribution was not normal, the Kruskal-Wallis multiple-comparison test was used. For comparing IPAA patients to the controls, the Mann-Whitney U-test was used. Correlation coefficients between two parameters were calculated with Spearman`s rank correlation. A comparison of incidences was performed with  $\chi^2$  statistics or Fisher`s exact test. Simple and multiple linear and stepwise regression analyses were performed to examine the effects of various independent factors. Any P value  $< 0.05$  was considered statistically significant. All statistical analyses were performed with NCSS 2000 software for Windows (NCSS statistical software, Kaysville, UT, USA).

## RESULTS

### 1. Incidence of pouchitis

During the mean 7.5 years (range 5-12 years) of follow-up, 46 (43%) of the 107 IPAA patients were treated once or more for clinical symptoms indicating pouchitis. Of the 61 patients without any acute episode of pouchitis, 16 (26%) achieved by PDAI scoring criteria a total score  $\geq 7$  (range 7-10), indicating ongoing pouchitis. Thus, pouchitis occurred in 62 (58%) of the patients during the follow-up period. Of the 107 patients, 15 (14%) exhibited macroscopic (friability, erythema, ulcers) and/or histologic inflammatory changes (PMN cell infiltration, ulcers, atrophy) in the proximal ileum above the pouch. This subgroup of patients had the highest inflammatory activity in the pouch as measured by PDAI: mean  $10.2 \pm 0.8$  (range 5 - 13), 95% CI 8.5 to 11.9. Ten patients (9.3%) had chronic symptoms requiring recurrent antibiotic therapy to keep the pouchitis in remission; however, 8 of these had a PDAI  $\geq 7$  (mean 9.9, range 5-14), indicating ongoing pouchitis and suggesting poor response to antibiotics used. Chronic pouchitis led to pouch excision in one male patient after his study participation. Of the 107 patients, 14 (13%) fulfilled our criteria of an optimal outcome: no history of pouchitis, no clinical symptoms, normal F/U endoscopies, no ulceration or acute polymorphonuclear leukocyte infiltration (PMN) in histological specimens during the follow-up.

### 2. Factors associated with mucosal inflammation

#### 2.1. *Previous course of ulcerative colitis*

Patients with pouchitis had a significantly shorter disease duration of UC than did those without pouchitis before surgery, which suggests their more aggressive and active UC inflammation (Study V) (Figure 2). The subgroup of 15 patients with proximal ileal involvement had taken a higher cumulative (mean  $9.2 \pm 1.9$ ; 95% CI 5.1 to 13.3 g versus  $4.4 \pm 0.7$ ; 95% CI 2.8 to 5.9 g,  $p = 0.07$ ) and daily (mean  $8.0 \pm 2.5$ ; 95% CI 2.4 to 13.6 mg versus  $2.7 \pm 0.9$ ; 95% CI 0.7 to 4.7 mg,  $p = 0.053$ ) steroid dose than had those with optimal outcome (Study IV). No differences between these two subgroups

appeared in the type of operation, extent of UC (left-sided/pancolonic) or indication (fulminant/chronic active, dysplasia) for surgery. Daily corticosteroid dose in mg of prednisone correlated positively with PDAI ( $r=0.26$ ,  $p = 0.026$ ) and negatively with age at operation ( $r= -0.25$ ,  $p = 0.027$ ). In a stepwise regression analysis that included gender, age at diagnosis of ulcerative colitis, daily corticosteroid dose in mg of prednisone, duration of UC in years, and extent of UC before surgery, the number of acute exacerbations of pouchitis was negatively correlated with duration of UC ( $p = 0.003$ ), and with age at diagnosis of UC ( $p = 0.017$ ).

**Table 8. Demographic characteristics of 107 UC patients with ileal pouches**

	Pouchitis, N = 62	No pouchitis N = 45	p
Sex, M/F	34/28	20/25	0.29
Mean age, yrs	44.5 ±1.3	46.0 ±1.6	0.30
Time since IPAA, yrs	7.8 ± 0.3	7.0 ± 0.3	0.05
Duration of UC, yrs	5.8 ± 0.6	8.8 ± 1.0	0.01
Extent of UC, pancolitis : left-sided	51/11	31/14	0.10
Indication for surgery			
fulminant: chronic	15/47	6/39	0.16
dysplasia (%)	9 (14)	4(9)	0.38
Operation type,			
one-stage : two-stage	35/27	25/20	0.93
Number with family history of IBD (%)	13 (21)	8(18)	0.68
Number with proximal ileum involvement after IPAA (%)	15 (24)	0	< 0.001

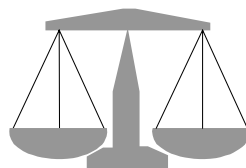
Values are numbers of patients or means ± SEM;

$p < 0.05$  was considered significant, Mann-Whitney U test,  $X^2$  -test.

**Figure 2. Influence of factors in the course of ulcerative colitis on long-term outcome after IPAA**

### Optimal outcome

- Longer disease duration of UC
- Lower daily and cumulative corticosteroid doses



### Pouchitis

- Shorter disease duration of UC
- Higher daily and cumulative corticosteroid doses
- Young age

## 2.2. Bacteria

Bacteriological analyses of fresh faecal samples and tissue biopsy specimens were run for 21 patients with a pouchitis history (PDAI  $\geq 7$  in previous follow-up endoscopy), and 11 patients with optimal outcome after IPAA.

The pouches of patients with pouchitis harboured increased concentrations of faecal anaerobes and aerobes with trend toward a reduced ratio of anaerobes to aerobes. This is reflected in the higher frequency of culture-positive samples of aerobes in patients with inflamed pouches than in patients with optimal outcome, who more often harboured anaerobic species, especially *Bacteroides* spp. Concentration of *Esheririchia coli* in faecal samples ( $p < 0.05$ ) and frequency of *Esheririchia coli*-positive biopsy samples in pouch ( $p < 0.05$ ) and ileum mucosa ( $p = 0.05$ ) was significantly higher in patients with inflamed pouches. No differences existed in Bifidobacterium and anaerobic Lactobacillus species between these groups. The main differences between the groups in total anaerobes and aerobes are presented in Table 9, and frequencies of bacteria for study groups in Table 10.

**Table 9. Microbiological assessment of tissue and faecal samples from patients with normal (n=11) and inflamed (n=21) pouches**

Log <sub>10</sub> CFU/g	Optimal outcome n = 11	95% CI	Pouchitis n = 21	95% CI	p
Proximal ileum					
Total anaerobes	3.7 ± 0.3	3.0 – 4.4	4.1 ± 0.2	3.7 – 4.5	0.41
Total aerobes	2.9 ± 0.1	2.6 – 3.1	3.1 ± 0.2	2.7- 3.5	0.57
Pouch mucosa					
Total anaerobes	4.1 ± 0.3	3.4 – 4.8	4.0 ± 0.2	3.5 – 4.4	0.65
Total aerobes	3.1 ± 0.2	2.5 – 3.6	3.4 ± 0.2	3.0 – 3.8	0.18
Faeces					
Total anaerobes	8.7 ± 0.2	8.2 – 9.2	9.4 ± 0.1	9.2 – 9.7	0.007
Total aerobes	7.0 ± 0.3	6.4 – 7.6	8.0 ± 0.2	7.6 – 8.3	0.007
Total anaerobes/ aerobes	1.3		1.2		0.14

Mean count expressed as log<sub>10</sub> CFU per gram of tissue ± SEM.

$p < 0.05$  was considered significant, Mann-Whitney U-test. CI: confidence interval

**Table 10. Frequency and mean count of bacteria for patients with optimal (n=11) and patients with inflamed pouches (n=21)**

Organism	% of samples (mean count of bacteria, CFU per gram of tissue) by group					
	Faeces		Pouch mucosa		Ileum mucosa	
	Optimal	Inflamed	Optimal	Inflamed	Optimal	Inflamed
<b>Aerobes</b>						
<i>Escherichia coli</i>	55 (4.7x10 <sup>6</sup> )	90* (1.3x10 <sup>8*</sup> )	18 (1.9x10 <sup>3</sup> )	57* (2.7x10 <sup>3</sup> )	9 (9.1x10 <sup>1</sup> )	43* (9.7x10 <sup>2</sup> )
Enterococci	45 (3.7x10 <sup>6</sup> )	62 (4.5x10 <sup>7</sup> )	27 (8.2x10 <sup>2</sup> )	24 (5.4x10 <sup>2</sup> )	9 (1.8x10 <sup>1</sup> )	30 (5.0x10 <sup>2</sup> )
Streptococci	55 (3.3x10 <sup>6</sup> )	67 (8.7x10 <sup>7</sup> )	18 (5.5x10 <sup>1</sup> )	38 (2.7x10 <sup>3</sup> )	27 (3.1x10 <sup>2</sup> )	40 (1.2x10 <sup>3</sup> )
<i>Staphylococcus</i> spp.	73 (7.7x10 <sup>4</sup> )	67 (1.9x10 <sup>7</sup> )	9 (9.1x10 <sup>2</sup> )	38 (7.0x10 <sup>2</sup> )	55 (3.2x10 <sup>2</sup> )	25 (4.8x10 <sup>2</sup> )
<b>Anaerobes</b>						
<i>Bacteroides fragilis</i> group	91 (1.0x10 <sup>9</sup> )	71 (2.2x10 <sup>9</sup> )	82 (2.0x10 <sup>4</sup> )	52 (2.0x10 <sup>4</sup> )	73 (9.6x10 <sup>3</sup> )	45 (1.8x10 <sup>4</sup> )
<i>Bacteroides vulgatus</i>	46 (2.9 x10 <sup>8</sup> )	33 (5.5 x10 <sup>8</sup> )	36 (2.0 x10 <sup>4</sup> )	19 (1.1 x10 <sup>4</sup> )	36 (7.8 x10 <sup>2</sup> )	14 (1.5 x10 <sup>3</sup> )
<i>Bacteroides thetaiotaomicron</i>	18 (7.4x10 <sup>7</sup> )	28 (5.0x10 <sup>8</sup> )	28 (4.6 x10 <sup>2</sup> )	36 (3.3 x10 <sup>4</sup> )	18 (4.1 x10 <sup>2</sup> )	20 (5.4 x10 <sup>3</sup> )
<i>Bacteroides ovatus</i>	46 (3.1 x10 <sup>7</sup> )	14 (8.6 x10 <sup>7</sup> )	27(3.1 x10 <sup>4</sup> )	0*	18(2.3 x10 <sup>7</sup> )	0*
Bifidobacteria	91 (2.7x10 <sup>8</sup> )	95 (8.4x10 <sup>8</sup> )	27 (4.8x10 <sup>3</sup> )	52 (6.4x10 <sup>3</sup> )	64 (2.8x10 <sup>3</sup> )	45 (3.7x10 <sup>3</sup> )
Lactobacilli	91 (1.7 x10 <sup>8</sup> )	90 (1.3 x10 <sup>8</sup> )	45 (3.5 x10 <sup>3</sup> )	38 (3.0 x10 <sup>3</sup> )	36 (1.1 x10 <sup>3</sup> )	35 (1.6 x10 <sup>3</sup> )

\* p ≤ 0.05, Mann-Whitney U test, X<sup>2</sup> -test.

### 2.3. Perinuclear antineutrophil cytoplasmic antibodies (pANCA)

The prevalence of pANCA in 107 IPAA patients was 30% (n = 32), and 28% in the 21 patients with conventional ileostomies. Of IPAA patients, 10 had high serum pANCA levels (> 100): 8 of these had pouchitis (PDAI  $\geq$  7, p = 0.036). In patients with high pANCA levels, the mean PDAI score was significantly higher  $8.7 \pm 1.2$  than in patients with low  $\leq$  100 (mean PDAI  $6.0 \pm 0.8$ ) or those with negative levels ( $4.9 \pm 0.9$ , p = 0.019).

### 2.4. Extraintestinal manifestations (EIMs)

EIMs were present before restorative proctocolectomy in 27 (25%) of the 107 IPAA patients, of whom 5 had more than one EIM (Table 11). Peripheral arthritis was the most common manifestation, and no differences appeared in rates of EIMs by sex. Analysis of patients with preoperative EIMs demonstrated that EIMs were a significant determinant of pouchitis disease activity. In particular, an increased risk for pouchitis occurred in patients with pre-operative ankylosing spondylitis (AS) or iritis: Of 14 such patients, 12 (86%) had pouchitis (PDAI  $\geq$  7); OR 11.4 (95%CI 2.3-39.3), p < 0.001. One patient had both pre-operative complications.

**Table 11. Prevalence of pouchitis in patients with history of extraintestinal manifestations (EIMs) before surgery for ulcerative colitis**

EIM	N	Number of patients with PDAI $\geq$ 7 (%)	OR (95% CI)	p
All EIMs	27	16 (59)	2.7 (1.1–6.4)	0.03
PSC	2	1 (50)	1.4 (0.1-14.3)	0.80
AS/ Sacroileitis	8	7 (88)	11.7 (1.4–50.4)	0.006
Peripheral arthritis	13	4 (31)	0.60 (0.2–3.5)	0.34
Iritis	7	6 (86)	9.8 (1.2–43.4)	0.013
Erythema nodosum	3	1 (33)	0.70 (0.1–6.6)	0.78

PSC, primary sclerosing cholangitis; AS, ankylosing spondylitis; OR, odds ratio; CI, confidence interval,  $\chi^2$ -test

## 2.5. Smoking

Smoking data were collected by questionnaire, or direct interview, or both. Current smokers showed a trend toward lower inflammatory activity and prevalence of pouchitis (Table 12).

**Table 12. Smoking habits, inflammation activity, and prevalence of pouchitis (PDAI  $\geq$  7)**

	Number	PDAI*	Pouchitis %**
Non-smoker	40	6.6 $\pm$ 0.6	50
Ex-smoker	36	4.9 $\pm$ 0.6	41
Smoker	31	4.6 $\pm$ 0.7	29

Values presented as number of patients or as means  $\pm$  SEM

\*p= 0.07, (ANOVA), \*\*p= 0.23 ( $\chi^2$ -test)

In a stepwise regression analysis that included sex, age, time since IPAA surgery, extent of UC before surgery, smoking habits, serum pANCA level, and occurrence of EIMs, pouchitis disease activity (PDAI) was correlated positively with serum pANCA levels (p = 0.005) and EIMs (p = 0.01), but negatively with smoking habits (p = 0.04).

## 3. Factors associated with mucosal morphology

### 3.1. Villous atrophy

Villous atrophy correlated positively with inflammatory activity (PDAI) (r= 0.67, p<0.001) and with number of total faecal anaerobes (r= 0.45, p= 0.01) and aerobes (r= 0.30, p= 0.09). All 21 patients with a conventional ileostomy showed normal villous architecture and had no inflammation. In stepwise and multiple regression analysis, number of pouchitis episodes and acute inflammation (infiltration of neutrophils) in the mucosa were both statistically significant predictors of the presence of villous atrophy. No correlation existed between degree of villous atrophy and age, sex, follow-up time, BMI, or smoking.

### 3.2. Colonic metaplasia

Some degree of sulphomucin in mucin staining occurred in 54 (50%) of 107 IPAA patients.

Associations between bacteria, dietary factors, faecal bile acids, and mucosal morphology were studied in 21 IPAA patients with pouchitis and in 11 patients with optimal outcome. Of 32 patients, 14 showed sulphomucin predominance. No differences existed in mean nutrient intake, composition of faecal bile acids, or microbial cultures of tissue biopsy between groups with and without pouchitis. Degree of colonic metaplasia was positively associated with faecal anaerobic ( $r= 0.44$ ,  $p= 0.01$ ) and aerobic flora ( $r= 0.42$ ,  $p= 0.02$ ). Low intake of lactose was associated with sulphomucin predominance. A negative correlation existed between faecal aerobes and dietary lactose consumption ( $r = -0.45$ ,  $p= 0.02$ ) (Table 13).

**Table 13. Clinical characteristics of 32 IPAA patients with sialomucin or sulphomucin predominance**

	Mucin predominance		p
	Sialomucin (n = 18)	Sulphomucin (n = 14)	
Sex, M/F	9/9	9/5	0.42
Time since IPAA, yrs	8.4 ± 0.6	8.8 ± 0.7	0.67
Duration of ulcerative colitis, yrs	8.0 ± 1.5	6.6 ± 1.5	0.58
PDAI	3.4 ± 0.9	7.6 ± 1.0	0.01
Subtotal/ total villous atrophy (%)	2 (11)	9 (64)	0.004
Bacteria, Log <sub>10</sub> CFU/g of tissue			
Faecal anaerobes	8.9 ± 0.16	9.6 ± 0.14	0.007
Faecal aerobes	7.4 ± 0.22	8.0 ± 0.23	0.07
Diet			
Dairy products (g)	327 ± 58	155 ± 38	0.04
Lactose (g)	16.7 ± 2.9	8.0 ± 1.9	0.02
Faecal bile acids			
Cholic acid (CA)	43.0 ± 7.8	37.9 ± 4.3	0.56
Chenodeoxycholic acid (CDCA)	17.2 ± 3.0	23.9 ± 2.1	0.07
Deoxycholic acid	7.8 ± 5.0	5.5 ± 2.0	0.71
Lithocholic acid	1.7 ± 1.0	2.0 ± 0.8	0.43

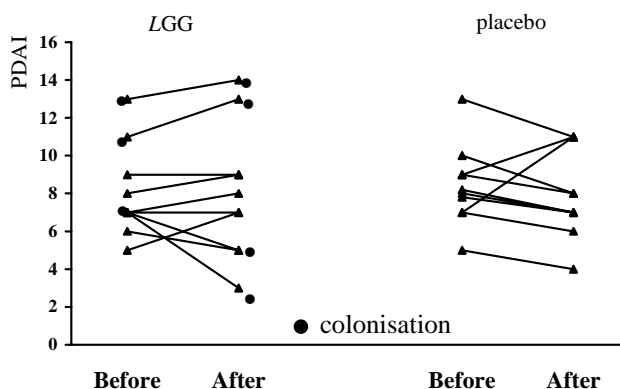
Values presented as means ± SEM,  $P < 0.05$  was considered significant, Mann-Whitney  $U$  test.

In a logistic regression analysis that included sex, time since surgery, number of total faecal aerobes and anaerobes, dietary fibre, intake of lactose, and primary bile acids, sulphomucin predominance (colonic metaplasia) was positively correlated with total faecal anaerobes ( $p = 0.03$ ), and negatively with lactose intake ( $p = 0.03$ ).

#### 4. Effect of *Lactobacillus* GG supplementation on pouch microbial flora and inflammation

Twenty patients (10 LGG, 10 placebo) with a previous history of pouchitis and endoscopic inflammation received *Lactobacillus* GG in gelatin capsules ( $0.5-1 \times 10^{10}$  cfu/capsule) or placebo, two capsules b.i.d for 3 months. *Lactobacillus* GG supplementation balanced the pouch intestinal flora by increasing the ratio of faecal total lactobacilli to total faecal anaerobes ( $p = 0.03$ ), to total faecal aerobes ( $p = 0.02$ ), and increasing the frequency of lactobacilli-positive cultures in pouch and afferent limb mucosa biopsy samples. However, only 40% of the patients were colonised with the *Lactobacillus* GG. No differences appeared between the groups in the PDAI score or in faecal or tissue biopsy samples of total anaerobes or aerobes. The effect of *Lactobacillus* GG supplementation on inflammation activity is presented in Fig 3.

**Figure 3. PDAI scores before and after *Lactobacillus* GG (LGG) or placebo supplementation**



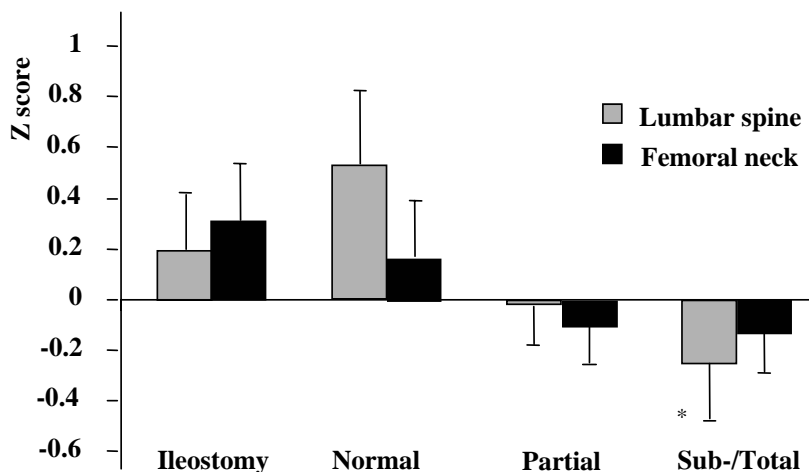
## 5. Factors associated with long-term metabolic consequences

### 5.1. Villous atrophy

A significant change toward lower absorption of bile acids, and lower serum levels of lipids, fat-soluble vitamins, and albumin occurred in patients with subtotal to total villous atrophy. The effect of villous atrophy on absorption studies and on long-term haematological and metabolic consequences is presented in Tables 14 and 15.

Prevalence of osteopenia (Z-score < -1) was 32%, but severe osteopenia or osteoporosis (Z < -2) was uncommon, found in only 2.3 % of IPAA patients. Those with osteopenia were significantly younger (mean age 33.5 yr  $\pm$  1.9; 95% CI 29.4 to 37.6 versus 40.9 yr  $\pm$  1.3; 95% CI 38.3 to 43.5) and had a lower body mass index (21.8 kg/m<sup>2</sup>  $\pm$  0.8; 95% CI 20.4 to 23.2 versus 24.9 kg/m<sup>2</sup>  $\pm$  0.5; 95% CI 23.7 to 26.1) at the time of the operation than did those with normal bone density. No differences appeared in mean cumulative or daily steroid dose. Effect of villous atrophy on bone mineral density at the lumbar spine and femoral neck is presented in Fig 4.

**Figure 4.** Lumbar spine (L1-L4) and femoral neck BMD Z scores in ileostomy patients (n=20) and in IPAA patients with normal villous structure (n=14), partial (n=47), or sub-/total villous atrophy (n=27). Means ( $\pm$  SE) of Z scores. P\* = 0.02 compared to patients with normal villous structure.



**Table 14. Haematological and absorption studies**

	Patients with IPAA, degree of villous atrophy				Significance
	Ileostomy	1. Normal	2. Partial	3.Subtotal/Total	
ESR, mm/h	11.7 ± 3.1	14.0 ± 3.5	13.0 ± 2.0	19.0 ± 2.5	p <sup>1</sup> 0.06 p <sup>2</sup> 0.17
Hb, g/l	144.0 ± 2.9	144.1 ± 3.1	141.2 ± 1.9	137.1 ± 2.2	0.08
Albumin, g/l	42.0 ± 0.8	43.2 ± 0.9	42.7 ± 0.5	40.5 ± 0.6	0.02
Calcium, mmol/l	2.37 ± 0.02	2.41 ± 0.02	2.36 ± 0.01	2.33 ± 0.01	0.002
Folate, nmol/l	19.6 ± 1.9	19.2 ± 2.0	20.7 ± 1.2	20.6 ± 1.4	0.65
Vitamin B <sub>12</sub> , pmol/l	416.7 ± 34.6	446.5 ± 37.5	328.8 ± 21.5	357.1 ± 27.8	0.15
Absorption studies					
Vit. B <sub>12</sub> - abs. %	26.0 ± 1.8	17.2 ± 1.9	19.4 ± 1.0	17.7 ± 1.4	0.98
SeHCAT/72h%	27.9 ± 4.0	30.5 ± 4.4	23.6 ± 2.3	20.1 ± 2.9	0.14

Values presented as means ± SEM, p < 0.05 was considered as significant, p<sup>1</sup> = comparison between groups 3 and 1. p<sup>2</sup> = comparison between group 3 and ileostomy.

**Table 15. Serum lipids and fat soluble vitamins**

	Patients with IPAA, degree of villous atrophy				Significance
	Ileostomy	1. Normal	2. Partial	3.Subtotal/Total	
Serum concentrations					
Cholesterol, mmol/l					
Total	5.50 ± 0.2	5.34 ± 0.2	5.02 ± 0.2	4.56 ± 0.2	0.015
LDL	3.06 ± 0.2	3.0 ± 0.2	2.73 ± 0.1	2.48 ± 0.2	0.06
HDL	1.92 ± 0.1	1.69 ± 0.1	1.66 ± 0.1	1.63 ± 0.1	0.08
Triglycerides, total	1.21 ± 0.2	1.47 ± 0.2	1.43 ± 0.1	1.00 ± 0.1	0.02
Vitamin A, µmol/l	2.4 ± 0.2	2.4 ± 0.2	2.3 ± 0.1	2.1 ± 0.1	0.16
Vitamin E, µmol/l	35.2 ± 1.8	34.3 ± 1.9	32.4 ± 1.2	26.9 ± 1.5	0.008
S-25OHD <sub>3</sub> , nmol/l	46.5 ± 4.5	52.4 ± 4.8	43.5 ± 2.9	42.8 ± 4.8	0.39

Values presented as means ± SEM, p < 0.05 was considered as significant, p<sup>1</sup> = comparison between groups 3 and 1. p<sup>2</sup> = comparison between group 3 and ileostomy.

## 5.2. Extent of inflammation in the remaining ileum

Patients with inflammation in the proximal ileum (n=14, Study I) displayed the lowest serum total cholesterol (mean 3.8 mmol/l  $\pm$  0.3,  $p < 0.001$ ), LDL (mean LDL 2.0 mmol/l  $\pm$  0.2,  $p = 0.002$ ), and HDL (mean 1.4 mmol/l  $\pm$  0.1,  $p = 0.009$ ) levels compared to levels of controls and patients with normal pouch morphology. Moreover, they showed the highest prevalence of osteopenia (66.7%) and the lowest spine (mean  $-0.89 \pm 0.36$ ,  $p = 0.006$ ) and femoral neck (mean  $-0.63 \pm 0.29$ ,  $p = 0.07$ ) Z scores.

## 6. Long-term functional outcome and patient satisfaction

A great majority of patients (90%) in both study groups were satisfied (Table 16). However, patients with IPAA tended to have daily more often intestinal symptoms. Occasional incontinence was reported by 55% of IPAA patients; 25% of these needed protective pads.

**Table 16. Prevalence of symptoms, changes in daily social activities, sexual function, and dietary restrictions in patients with conventional ileostomy (n = 21) and with IPAA (n = 104)**

Variable	Ileostomy (%)	IPAA (%)	p
Overall satisfaction	19 (90)	93 (89)	
Intestinal symptoms			
no symptoms	13 (62)	40 (39)	
occasionally	7 (33)	42 (41)	
daily/ severe	1 (5)	21 (20)	0.09
Social activity			
changes with social interaction	6 (29)	16 (15)	0.15
avoidance of special social happenings	18 (86)	33 (32)	<0.001
change in work	3 (14)	14 (14)	0.95
hobbies	6 (29)	16 (16)	0.15
travelling	5 (24)	26 (25)	0.89
Sexual function			
impotence	2 (22)	7 (14)	0.50
retrograde ejaculation	1 (11)	3 (6)	0.55
dyspareunia	3 (30)	10 (21)	0.55
Dietary restriction	6 (29)	30 (29)	0.99

Values are presented as numbers,  $p < 0.05$  was considered as significant,  $X^2$ -test

## DISCUSSION

### 1. Incidence of pouchitis

The main complication after restorative proctocolectomy with ileal pouch-anal anastomosis remains pouchitis. The latest reported rates of incidence of pouchitis in patients operated on for ulcerative colitis range from 29 to 59% (Simchuk *et al.* 2000, Heuschen *et al.* 2001a). Our rate of pouchitis of 58% falls within this range.

To minimise selection bias, the present study population comprised of consecutive patients operated on for ulcerative colitis between the years 1985 and 1994 at the Department of Surgery of Helsinki University Central Hospital. Of 150 invited patients living in the main catchment area of our hospital, 110 expressed their interest in participating. Those willing to participate in any such study may have more symptoms, in this case a higher inflammation score. However, a recent study by Shen *et al.* has shown that 25% of patients with a high symptom score suggestive of pouchitis did not fulfil criteria for the diagnosis of pouchitis as measured by PDAI. In addition, endoscopic and histologic inflammation are not always associated with the presence of severe symptoms (Luukkonen *et al.* 1994, Shen *et al.* 2001a), a finding compatible with the present findings. Of patients without any pouchitis history, 26% had significant endoscopic and histologic inflammation and achieved a PDAI score of 7 or higher, indicating active pouchitis. This finding compares well with that of Shen *et al.* (2001a), who reported that 36% of patients with only mild symptoms not typically suggesting the diagnosis of pouchitis had  $PDAI \geq 7$ . Thus, a combination of symptoms and endoscopic and histologic evaluations should always be pursued for accurate diagnosis of pouchitis. For a considerable number of patients, defining pouchitis merely on the basis of clinical symptoms would mean a risk of overlooking chronic undetected inflammation. This suggests the need for long-term surveillance of all patients with IPAA, as proposed by Shepherd (1990). If patients are seen only on a nonroutine basis, only when experiencing symptoms of pouchitis, pouchitis incidence would seem unrealistically low.

Although the number of control subjects with conventional ileostomies was limited, and they underwent surgery before the era of IPAA, and were of higher mean age with a longer follow-up time, in all of them, prestomal ileal mucosa was normal.

## 2. Severity and extent of inflammation

The pouchitis disease activity inflammation (PDAI) score allows differentiation only between “no pouchitis” (< 7 points) and “pouchitis” (≥ 7 points). This strict criterion may be misleading, because inflammation activity varies greatly among patients and is not an “on-off” phenomenon. Active and chronic inflammation may still exist in the majority of those with PDAI < 7, “no pouchitis”. Some degree of pathological inflammation can be found endoscopically and histologically in almost every pouch. This low inflammation activity has been seen as an adaptive inflammation, because it is almost universal (Heuschen *et al.* 2001a). It is suggested that this form of inflammation has little or no impact on a patient’s well-being and long-term outcome after IPAA. However, Thompson-Fawcett and colleagues presented a case of a 37-year-old woman who had dysplasia with inflammation activity at only a low acute and chronic level (Thompson-Fawcett *et al.* 2001).

An optimal outcome with no inflammation appeared in 13% of our IPAA patients after a mean 7.5-year follow-up. This subgroup of patients had a slightly longer disease duration of UC, and their cumulative dose of corticosteroid used before colectomy was lower, suggesting a more benign disease course.

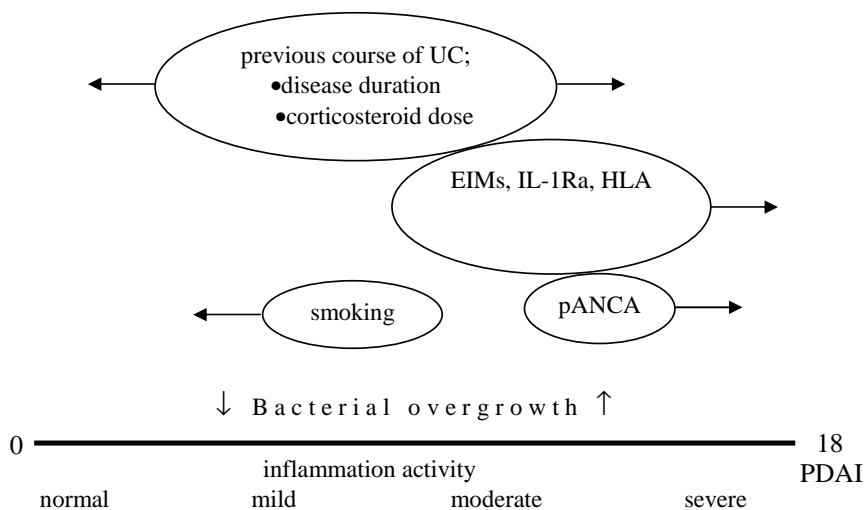
The high inflammatory activity with proximal ileal involvement in 14% of our patients suggests ongoing chronic active pouchitis. A major concern in this subgroup of 15 patients is the risk for unrecognised Crohn’s disease. These patients showed the highest pouch-inflammation levels in our study. Inflammatory activity in the proximal limb was restricted, usually distally to less than 10 cm above the pouch junction. Endoscopically, no deep ulcers or granulomas in histological specimens were evident, suggesting that inflammatory activity in the proximal limb occurs as a manifestation of chronic primary pouchitis rather than indicating a misdiagnosis of the original disease process.

### 3. Factors associated with severity of inflammation

#### 3.1. Previous course of ulcerative colitis

The extent of preoperative UC in the colon was not a risk factor for development of pouchitis. In previous studies this question has aroused controversy. Our data parallel those of Samarasekera *et al.* (1996), who found no relationship between distal colitis or more extensive disease and frequency of pouchitis. In contrast, Schmidt *et al.* (1998) reported that the colonic extent of the disease had a significant association after IPAA with subsequent development of pouchitis; severity of the UC pre-operatively was not predictive for pouchitis, unlike in our findings (Figure 5).

**Figure 5. Factors associated with postoperative ileal inflammation activity in pouches**



Our data show (Studies II, III, V) that the severity of the post-operative inflammatory process is associated with severity of the UC. Patients with pouchitis had a significantly shorter disease duration of UC than did those without pouchitis. Their pre-operative cumulative and daily corticosteroid doses had been significantly higher, suggesting

more aggressive and active UC inflammation. It is possible that such patients are immunologically or genetically more susceptible to ulcerative colitis and thus to pouchitis than are those with an optimal outcome (Study II). Carter and colleagues (2001) reported that the interleukin 1 receptor antagonist gene allele 2 predicts pouchitis after IPAA in ulcerative colitis.

Our patients with extraintestinal manifestations before surgery had about a 3-fold risk for developing pouchitis. Pouchitis (PDAI $\geq$  7) occurred in 59% of the patients at risk, a finding comparable to findings of studies reporting a prevalence of pouchitis of 61% to 64% in patients with EIMs (Lohmuller *et al.* 1990, Penna *et al.* 1996). Studies have revealed that factors leading to active inflammation show an overlap among IPAA, UC, and EIMs. In ulcerative colitis, a strong association has been described between disease behaviour and HLA allelic variation. It has become apparent that the HLADR B1\*0103 allele may predict severity of UC, need for surgery, and presence of EIMs involving the joints, skin, and eyes (Roussomoustakaki *et al.* 1997, Orchard *et al.* 2000). HLA associations with primary sclerosing cholangitis (PSC) also exist: the HLA-B8/DR3 haplotype is frequent in patients with UC with PSC but infrequent in patients with UC without PSC (van Milligen de Wit *et al.* 1995).

### **3.2. pANCA**

In UC, pANCA expression is associated with distinct clinical phenotypes, including treatment-resistant left-sided disease (Sandborn *et al.* 1996), aggressive disease course (Vecchi *et al.* 1998), and a need for surgical intervention early in the disease course (Boerr *et al.* 1995). Here we show that, of those with high pANCA levels (> 100) after colectomy, 80% have pouchitis. Studies examining the correlation between pANCA expression and pouchitis have been contradictory. Yasuda *et al.* (1998) found no significant difference in pANCA expression between patients with or without pouchitis. Sandborn *et al.* (1995) found a frequency of pANCA in patients with chronic pouchitis of 100% compared with only 50% in patients without pouchitis. A recent study by Fleshner *et al.* (2001) showed that a high level of pANCA before colectomy was significantly associated with development of chronic pouchitis. Produced by mucosal B-

cells, pANCA reflects a disease activity-associated immune response; the immunoreactive protein epitopes are expressed by colonic bacteria (e.g., *Bacteroides caccae*, *Escherichia coli*) (Cohavy *et al.* 2000). It is possible that patients with high levels of pANCA are predisposed to develop a more vigorous immune response to bacterial products.

### **3.3. Smoking**

Current smokers had a lower prevalence of pouchitis (29% vs. 50%) than did non-smokers. In a regression analysis model smoking was negatively correlated with inflammatory activity. This finding agrees with that of Merret *et al.* (1996) who found, after IPAA for UC, a strongly protective effect for current cigarette smoking against the development of pouchitis. It appears that the effect of smoking on pouchitis parallels that seen in UC (Thomas *et al.* 1998). What component of cigarette smoke is beneficial is unclear.

### **3.4. Bacteria**

The most dramatic finding concerned total counts in faecal bacterial samples from patients with pouchitis, which were significantly higher than those noted in the group of patients with an optimal outcome. This finding differs from earlier findings in subjects with optimal outcome or good results compared with subjects suffering inflammation. Sandborn *et al.* (1995) and O'Connell *et al.* (1986) reported no differences in anaerobic or aerobic bacteria between patients with and without pouchitis. Ruseler-van Embden *et al.* (1994) found an increased number of total faecal aerobes ( $p= 0.03$ ) but no difference in anaerobes. As no prior antibiotic medication was allowed, and none of our subjects were examined during any acute exacerbation of pouchitis, we thus assume that the microbial and morphological changes we found represent a stable and long-term condition in the pouch.

The diversity of isolated bacteria was high both in faecal and in mucosal biopsy samples with no differences found in mucosal concentrations of bacteria. Species isolated from the

mucosa were all of faecal origin, and the main anaerobic microorganism isolated from the mucosa was *Bacteroides*. The main aerobes were primarily *E. coli*. Similar to findings of Ruseler-van Emden *et al.* (1994), we found a trend toward a decreased ratio of anaerobes to aerobes in those with a history of pouchitis. This is reflected in the significantly higher concentration of *E. coli* in faecal samples, and in higher frequency in mucosal samples in patients with inflamed pouches than in those with an optimal outcome.

These findings are in parallel with Swidsinski *et al.* (2002), who reported high concentrations of *Bacteroides* and *E. coli* in mucosa biopsy samples in patients with active ulcerative colitis and Crohn's disease.

Many investigators have studied adhesive *E. coli* in IBD in humans. Based on an in-vitro adherence assay to epithelial cells, adhesive *E. coli* strains were found to be significantly more frequent in patients with IBD than in healthy controls, leading to the assumption that *E.coli* adherence may be a primary importance rather than arising secondary to the disease (Giaffer *et al.* 1992, Darfeuille-Michaud *et al.* 1998). However, serotyping data and analysis of genetic virulence markers of mucosal *E. coli* showed wide variability among mucosal *E. coli* strains, which speaks against a single *E. coli* pathogen's being responsible (Swidsinski *et al.* 2002).

Our microbial findings for faecal and tissue biopsy samples suggest that the total load of luminal bacterial flora, the faecal stream, and exposure to bacteria all play important roles in the aetiology of pouchitis. However, the high diversity of bacterial flora without a predominance of a single microorganism speaks against a specific pathogen's being the cause. It is evident that a specific host response, resulting from peculiarities of immunity, genetic disposition, or symbioses-like interaction, plays an important role in the aetiology of pouchitis.

#### **4. Effects of *Lactobacillus rhamnosus* GG on ileal-pouch inflammation**

In our study, a single-strain *Lactobacillus rhamnosus* GG administered in two gelatin capsules (0.5 – 1 x 10<sup>10</sup> cfu/capsule) b.i.d. for 3 months balanced faecal bacterial flora by elevating the ratio of total lactobacilli to total anaerobes and aerobes but was

inefficient as a primary therapy for clinical response. No differences appeared in the mean inflammation activity before or after supplementation.

In animal models, the effects of lactobacilli on intestinal inflammation have been controversial. Madsen *et al.* (1999) have shown that *Lactobacillus* sp. reduced levels of colonic mucosal adherent and translocated bacteria and attenuated development of colitis in interleukin 10 gene-deficient mice. A recent study by Holma and colleagues (2001) showed that *Lactobacillus rhamnosus* GG did not affect the severity of acute acetatic acid-induced colitis in the rat.

*Lactobacillus* GG was recovered in the faecal flora in 40% of the cases, but was not found in mucosal biopsies. No single factor predicting survival existed in a stepwise regression analysis. It is, however, evident that the fast transit time, high stool frequency (mean 7/day in our study), watery stool, and changes in mucosal morphology (villous atrophy) may disturb the adherence and colonisation of probiotic bacteria. As *Lactobacillus* GG is adherent to intestinal mucus, the bolus of bacteria ingested in capsules may be washed out, together with ileal secretions. It is evident that in patients with ileal-pouches, higher doses of probiotics may be needed for probiotics`intestinal survival when administration is in freeze-dried form.

## **5. Consequences of bacterial overgrowth and inflammation**

### ***5.1. Mucosal morphology***

A mucosal biopsy specimen from normal functioning pouches after IPAA shows villous atrophy, crypt hyperplasia, and change in mucin type from sialomucin to sulphomucin. These changes have been interpreted as an adaptation of the ileal mucosa to luminal changes (Moskowitz *et al.* 1986). In the present study, villous atrophy was strongly associated with inflammation activity in the pouch. Subtotal to total villous atrophy occurred most often in patients with chronic active pouchitis. Thus, in long-term follow-up, villous atrophy can be viewed as a simple marker of inflammatory activity rather than as an adaptive response in the pouch. In the long term, this may be important, because both chronic pouchitis and villous atrophy are connected to an increased risk for development of

dysplasia (Gullberg *et al.* 1997). However, the latest data so far suggest that development of dysplasia in ileal pouches is probably a rare event (risk < 0.05%) within 15 to 20 years of pouch surgery (Thompson-Fawcett *et al.* 2001).

A significant change in mucin staining towards a more colonic type of sulphomucin occurred in our patients with a pouchitis history, a finding closely similar to that of earlier studies (Shepherd *et al.* 1987, De Silva *et al.* 1991b, Luukkonen *et al.* 1994). The mechanisms underlying colonic metaplasia are likely to be influenced by interaction between bacteria, SCFA, and bile acids.

A novel finding was that our patients with sulphomucin predominance showed a trend toward reduced daily intake of dairy products. It is said that cross-sectional studies evaluating current diet are of little value, as nutritional habits are already influenced by the illness itself. Most of the patients reported, however, that they had continued their usual diets after colitis surgery.

Lactose intake, reflecting consumption of dairy products, was significantly inversely correlated, in a multiple and logistic regression analysis, with sulphomucin predominance. Our study sample is small, but this association is interesting, because lactose has been seen as a potential prebiotic factor. In inflammatory bowel disease, lactose may promote growth of the lactic acid-producing bacteria and thus prevent the expansion of potential pathogens (Szilagyi 2002). We found a negative correlation between lactose and faecal aerobes. Earlier studies have shown that regular lactose consumption in dairy products causes a trend toward lower levels of intestinal microbial flora (Ito and Rimura 1993).

### ***3.2. Is pouchitis a metabolic problem?***

We have shown that patients with IPAA more often have metabolic consequences than do patients with conventional ileostomies, who preserve their normal villous architecture and have no inflammation. Chronic and acute inflammation with ulceration in the pouch leads to villous atrophy and to loss of absorptive capacity and changes in pouch permeability and ileal function. These changes are associated with metabolic consequences.

Although hypoalbuminemia was rare (< 5%), serum albumin concentration presented a significant inverse correlation with inflammatory activity. Hypoalbuminemia is a very common finding in IBD, and results predominantly from intestinal protein loss and increased catabolism. Serum albumin is merely a marker of disease activity rather than of nutritional status (Geerling *et al.* 1999).

Our findings confirm the results of a study by Hakala *et al.* (1997), who reported impaired cholesterol absorption after IPAA. In long-term follow-up, however, lower lipid concentrations are evident, especially in patients with recurrent or chronic pouchitis and villous atrophy. Among our patients after IPAA, although vitamin D deficiency appeared in 10.6%, we were unable to demonstrate any significant correlation between serum 25(OH)D<sub>3</sub> and inflammation activity, villous atrophy, or bile acid absorption. The common use of multivitamins by the study subjects may be a confounding factor.

IPAA patients with subtotal to total villous atrophy have a higher prevalence of osteopenia than do subjects with normal villous structure. Our prevalence of osteopenia (32%) compares with earlier findings in IBD patients, although severe osteopenia or osteoporosis (Z score < -2) was uncommon, found in only 2.3% of IPAA patients. This may represent recovery of bone density after surgery for ulcerative colitis. The recent longitudinal study by Abitbol *et al.* (1997) showed a positive change in bone density at the spine (+2.3%) and femoral neck (+2.1%) during a mean 28-month follow-up after proctocolectomy for ulcerative colitis. In our subjects with a conventional ileostomy, normal villous architecture was preserved, but lumbar spine osteopenia was common (prevalence 30%) among them. One limitation of the present study was that patients with conventional ileostomy were significantly older, and the prevalence of postmenopausal women was the highest (35%), among all groups.

Inflammatory activity is usually restricted to the pouch, only occasionally rising into the proximal limb. Thus, the risk for developing hypoalbuminemia or hypovitaminosis seems to be less frequent in patients with IPAA than in patients with active Crohn's disease or ulcerative colitis. However, those patients with proximal ileum involvement are at significantly increased risk for long-term metabolic consequences. In them, pouchitis may be a metabolic problem.

#### **4. Life after colectomy**

The clinical and functional results after UC and conventional ileostomy and ileal pouch-anal anastomosis are well known: both are safe, both incur moderate morbidity, and both are efficacious. Quality of life following surgery for ulcerative colitis is excellent in most patients. Although the pelvic pouch procedure has become the choice for most patients, those with a conventional ileostomy appear to have an excellent quality of life, as well. This was evident also in our study. In terms of overall satisfaction, the patient groups were similar: 90% of conventional ileostomy and 89% of ileal pouch-anal anastomosis patients stated that they were satisfied. Limitations in work, diet, hobbies, and sexual life were equal in both groups. The most significant difference was that 86% of ileostomy patients reported an avoidance of special social happenings. Such happenings were not specified in the questionnaire. A shortcoming of our study was the questionnaire, which was non-validated, and the small number of control patients. However, our data compare well with those of other studies. Kohler *et al.* (1991) from the Mayo Clinic surveyed 406 patients who had a conventional ileostomy, 403 patients with a Koch pouch and 300 with a pelvic pouch and reported more than 90% in each of the three groups to be satisfied with their current status; more than 90% had returned to work. These patients were also similar in their ability to take part in social and recreational activities and sports, perform housework, enjoy family relationships and sexual activity, and travel. Jimmo and Hyman (1998) studied 12 patients with a conventional ileostomy and 55 with a pelvic pouch procedure. Overall, 83% of patients with a pelvic pouch and 83% with a conventional ileostomy were satisfied with the procedure. When patients completed the IBD questionnaire (IBDQ), no significant differences appeared between these two groups in overall score or by category.

#### **7. What are we really saving?**

Restorative proctocolectomy with ileal pouch-anal anastomosis was developed in an attempt to eliminate the need for a permanent stoma, to preserve the patients' body image, and thus to improve quality of life. However, our data and earlier reports show that in both operation groups over the long-term, quality of life was equal. Both

operations for treatment of ulcerative colitis have their advantages and their disadvantages and risks. The pouchitis syndrome is a major drawback after surgery. It is a bacteria-mediated disease in patients immunologically or genetically susceptible to UC. In the presence of bacterial overgrowth in the pouch, mechanisms involved in the etiopathogenesis of ulcerative colitis will strike back, leading to chronic pouchitis. Thus, considering only inflammation mechanisms, ileal pouch-anal anastomosis is a non-rational operation. Long-standing chronic mucosal inflammation in the gut with a small risk for neoplastic transformation have to be taken into consideration. Patients with chronic pouchitis will need long-term follow-up, and long-term medical treatment in lieu of a permanent ileostomy.

Patients who require surgery for ulcerative colitis have several options available. Considering postoperative morbidity and quality of life aspects, each operation should be carefully considered, and the pro and cons discussed in detail with the patient and his or her family.

## **8. Recommendations for long-term follow-up.**

Clinically, this study suggests that patients with extraintestinal manifestations and high level pANCA should be counselled on their high risk for developing chronic pouchitis after surgery. Of all cases of pouchitis in UC, 80 to 90% occur during the first 5 years after construction of the IPAA (Luukkonen *et al.* 1994, Heuschen *et al.* 2001). Cumulative risk of pouchitis ranges from 47 to 59% after 10 years following surgery. Absence of clinical symptoms, as shown also in the present study, is not a safe marker for absence of pouchitis.

According to previous and the present data, it is obvious that all patients should be re-evaluated 5 years after proctocolectomy to diagnose also those who have developed a silent, asymptomatic chronic inflammation. A limitation at the present time is the lack of any standard recommendations for long-term follow-up in the literature. I agree with Heuschen and colleagues at the University of Heidelberg who recommend standard long-term follow up after 5 years at 2-year intervals. Patients with chronic pouchitis should be surveyed in an intensified follow-up program.

## SUMMARY AND CONCLUSIONS

The aim of the present study was to study the long-term outcome after restorative proctocolectomy with ileal pouch-anal anastomosis, the factors associated with mucosal morphology and inflammation, and the influence of *Lactobacillus rhamnosus* GG on ileal inflammation and the microbial flora. Patients with conventional ileostomies served as a control group.

The main findings were as follows.

- Both villous atrophy and colonic metaplasia were positively associated with faecal anaerobic and aerobic flora. Villous atrophy can be seen as a simple marker of inflammatory activity in long-term follow-up. All patients with conventional ileostomies had normal mucosal morphology without inflammation.
- The incidence of pouchitis was 58% with increased risk for pouchitis in patients with extraintestinal manifestations before surgery and high pANCA levels after surgery. Current smokers tended to show a more benign disease course. Patients with pouchitis harboured significantly higher faecal counts of anaerobic and aerobic bacteria and a trend towards a lower ratio of total anaerobes to aerobes.
- Metabolic sequelae were associated with villous atrophy, mucosal inflammation, and extent of the disease in the remaining ileum and included decreased levels of albumin, calcium, total cholesterol, triglycerides, and vitamin E. Vitamin D and B<sub>12</sub> deficiencies also occurred.

- *Lactobacillus rhamnosus* GG balanced the bacterial flora by increasing the ratio of total lactobacilli to total anaerobes, but did not ameliorate mucosal inflammation.
- No differences appeared between the groups in quality of life. In terms of overall satisfaction the patient groups were similar: 90% of conventional ileostomy and 89% of ileal pouch-anal anastomosis patients were satisfied.

In conclusion, pouchitis is a bacteria-mediated disease in patients immunologically or genetically susceptible to ulcerative colitis. Patients with extraintestinal manifestations and high-level pANCA should be counseled on their high risk for chronic pouchitis after surgery and their potential need for long-term antibiotic treatment. Patients with chronic pouchitis will also require long-term follow-up. Patients with conventional ileostomies preserve better mucosal morphology and have excellent metabolic outcome and good quality of life. In the light of postoperative morbidity and the expected quality of life, surgeons should carefully consider both choices of operation and detail all pros and cons with patients and their families before surgery.

## ACKNOWLEDGEMENTS

This study was carried out at the Division of Gastroenterology at Helsinki University Central Hospital between 1997 and 2003.

I have been very fortunate to be supervised by Docent Martti Färkkilä, the Head of the Division of Gastroenterology at the Helsinki University Central Hospital. To him I owe my deepest gratitude and respect. Without his continuous support, optimism, and friendly guidance in my work, I would not have finished this study.

I extend my cordial thanks to Docents Seppo Niemelä and Martti Matikainen, the official reviewers of this thesis, for their valuable advice concerning the final manuscript.

I am very grateful to Professor Heikki Järvinen for his support and constructive criticism during the study.

I owe my sincerest thanks to Professor Arvi Kahri for performing the histological analyses of the ileal-pouch biopsy samples.

I wish to express my gratitude to Silja Mentula, M.Sc., and Professor Hannele Jousimies-Somer, from the Department of Microbiology at the National Public Health Institute for performing the microbial studies. I will long remember Hannele Jousimies-Somer, who died during these studies, as a lady of wit and sympathy. Her fabulous expertise in the field of microbial analyses is greatly appreciated.

I wish to thank my co-authors, Docent Pekka Luukkonen and Dr Hannu Nuutinen, M.D., Ph.D., for their co-operation.

I express my appreciation to Maija Saxelin, Ph.D., at Valio Ltd for her wonderful collaboration during the *Lactobacillus* GG supplementation study.

The aid of Carol Norris, Ph.D., in author-editing the language of the manuscript is gratefully acknowledged.

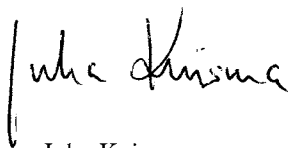
I thank my colleagues at the Division of Gastroenterology who gave me valuable advice and support during this task. I owe my warmest thank also to the study assistants and the staff. Their contribution was essential in completing this study.

The patients with ileoanal anastomosis as well as the control patients who were the focus of this study have all been extremely co-operative and have unfailingly subjected themselves to follow-up investigations. I offer them my warmest thanks.

This study was financially supported by a grant from the Finnish Foundation for Gastroenterological Research.

Finally, I wish to express my profound gratitude to my dear wife Raili, and to our children, Joonas and Julia, for their love and patience over the years of my commitment to this task.

Hyvinkää, December 2003

A handwritten signature in black ink, appearing to read 'Juha Kuisma'. The signature is written in a cursive style with a large initial 'J'.

Juha Kuisma

## REFERENCES

- Abitbol V, Roux C, Chaussade S, Guillemant S, Kolta S, Dougados M, Couturier D, Amor B. Metabolic bone assessment in patients with inflammatory bowel disease. *Gastroenterology* 1995;108:417-422.
- Abitbol V, Roux C, Guillemant S, Valleur P, Hautefeuille P, Dougados M, Couturier D, Chaussade S. Bone assessment in patients with ileal pouch-anal anastomosis for inflammatory bowel disease. *Br J Surg* 1997;84:1551-1554.
- Aggarwal BB, Puri RK. *Human Cytokines: Their role in disease and therapy*, BB Aggarwal, RK Puri, editors. Boston, Blackwell, 1994.
- Aitola P, Matikainen M, Mattila T, Tomminen T, Hiltunen KM. Chronic inflammatory changes in the pouch mucosa are associated with cholangitis found on peroperative liver biopsy specimens at restorative proctocolectomy for ulcerative colitis. *Scand J Gastroenterol* 1998;33:289-93.
- Aitola P, Miettinen A, Mattila A, Matikainen M, Soppi E. Effects of proctocolectomy on serum antineutrophil cytoplasmic antibodies in patients with chronic ulcerative colitis. *J Clin Pathol* 1995;48:645-647.
- Ambroze WL, Pemberton JH, Phillips SF, Bell AM, Haddad AC. Fecal short chain fatty acid concentrations and effect on ileal pouch function. *Dis Colon Rectum* 1993;36:235-239.
- Andersson RE, Olaison G, Tysk C, Ekblom A. Appendectomy and protection against ulcerative colitis. *N Engl J Med* 2001;344:808-814.
- Andreassen H, Rungby J, Dahlerup JF, Mosekilde L. Inflammatory bowel disease and osteoporosis. *Scand J Gastroenterol* 1997;32:1247-1255.
- Anton PA, Shanahan F. Neuroimmunomodulation in inflammatory bowel disease. How far from "bench" to "bed-side"? *Ann N Y Acad Sci* 1998;840:723-734.
- Ardizzone S, Bollani S, Bettica P, Bevilacqua M, Molteni P, Porro GB. Altered bone metabolism in inflammatory bowel disease: there is a difference between Crohn's disease and ulcerative colitis. *J Intern Med* 2000;247:63-70.
- Arend WP. Interleukin-1 receptor antagonist. *Adv Immunol* 1993;54:167-227.
- Asquith P, Mackintosh P, Stokes PL, Holmes GK, Cooke WT. Histocompatibility antigens in patients with inflammatory bowel disease. *Lancet* 1974;26:113-115.
- Awad RW, el-Gohary TM, Skilton JS, Elder JB. Life quality and physiological morbidity with an ileostomy. *Br J Surg* 1993;80:252-253.
- Bayat M, Brynskov J, Dige-Petersen H, Hippe E, Lonborg-Jensen H. Direct and quantitative vitamin B<sub>12</sub> absorption measurement in patients with disorders in the distal part of the bowel. Comparison of stool spot test [SST] with whole body counting in patients with ileal pelvic reservoir, ileostomy or Crohn's disease. *Int J Colorectal Dis* 1994;9:68-72.

- Bayly RJ, Bell TK, Waters A. A dual isotope modification of the Schilling test. In: Horst W, Pabst HQ, eds. *Ergebnisse der klinischen Nuklearmedizin*. Stuttgart, New York:FK Schattauer Verlag, 1971; 911-915.
- Belliveau P, Trudel J, Vasilevsky CA, Stein B, Gordon PH. Ileoanal anastomosis with reservoirs: complications and long-term results. *Can J Surg* 1999;42:345-352.
- Bengmark S. Ecological control of the gastrointestinal tract. The role of probiotic flora. *Gut* 1998;42:2-7.
- Bergstrand O, Hellers G. Breast-feeding during infancy in patients who later develop Crohn's disease. *Scand J Gastroenterol* 1983;18:903-906.
- Bernstein CN, Blanchard JF, Rawsthorne P, Yu N. The prevalence of extraintestinal diseases in inflammatory bowel disease: a population-based study. *Am J Gastroenterol* 2001;96:1116-1122.
- Bhagat S, Das KM. A shared and unique peptide in human colon, eye and joint detected by a novel monoclonal antibody. *Gastroenterology* 1994; 107:103-108.
- Bjarnason I, Macpherson A, Mackintosh C, Buxton-Thomas M, Forgacs I, Moniz C. Reduced bone density in patients with inflammatory bowel disease. *Gut* 1997;40:228-33.
- Boerr LA, Sambuelli AM, Katz S, et al. Clinical heterogeneity of ulcerative colitis in relation to frequency of pANCA reactivity. *Gastroenterology* 1995;108: A785.
- Breen EM, Schoetz DJ Jr, Marcello PW, Roberts PL, Collier JA, Murray JJ, Rusin LC. Functional results after perineal complications of ileal pouch-anal anastomosis. *Dis Colon Rectum* 1998;41:691-695.
- Brooke BN. The management of an ileostomy including its complications. *Lancet* 1952;2: 102
- Burke DA, Axon AT. Adhesive *Escherichia coli* in inflammatory bowel disease and infective diarrhoea. *BMJ* 1988 9;297:102-104.
- Calkins BM, Mendeloff AI. Epidemiology of inflammatory bowel disease. *Epidemiol Rev* 1986;8:60-91.
- Calkins BM. A meta-analysis of the role smoking in inflammatory bowel disease. *Dig Dis Sci* 1989;34:1841-1854.
- Carbonnel F, Boruchowicz A, Duclos B, Soule JC, Lerebours E, Lemann M, Belaiche J, Colombel JF, Cosnes J, Gendre JP. Intravenous cyclosporine in attacks of ulcerative colitis: short-term and long-term responses. *Dig Dis Sci* 1996;41:2471-2476.
- Carlstedt A, Fasth S, Hulten L, Nordgren A, Palselius I. Long-term ileostomy complications in patients with ulcerative colitis and Crohn's disease. *Int J Colorectal Dis* 1987;2: 22-25.
- Carter MJ, Di Giovine FS, Cox A, Goodfellow P, Jones S, Shorthouse J, Duff GW, Lobo AJ. The interleukin 1 receptor antagonist gene allele 2 as a predictor of pouchitis following colectomy and IPAA in ulcerative colitis. *Gastroenterology* 2001;121:805-811.

Casini-Raggi V, Kam L, Chong YJT, Focchi C, Pizarro TT, Cominelli F. Mucosal imbalance of IL-1 and IL-1 receptor antagonist in inflammatory bowel disease: a novel mechanism of chronic intestinal inflammation. *J Immunol* 1995;154:2434-2440.

Christie PM, Knight GS, Hill GL. Metabolism of body water and electrolytes after surgery for ulcerative colitis: conventional ileostomy versus J pouch. *Br J Surg* 1990;77: 149-151.

Church JM, Fazio VW, Lavery IC. The role of fiberoptic endoscopy in the management of the continent ileostomy. *Gastrointest Endosc.* 1987;33:203-9.

Clausen MR, Tvede M, Mortensen PM. Short chain fatty acids in pouch contents from patients with and without pouchitis after ileal pouch-anal anastomosis. *Gastroenterology* 1992;103:1144-1153.

Cohavy O, Bruckner D, Gordon LK, Misra R, Wei B, Eggena ME, Targan SR, Braun J. Colonic bacteria express an ulcerative colitis pANCA-related protein epitope. *Infect Immun* 2000;68:1542-1548

Cohen RD, Stein R, Hanauer SB. Intravenous cyclosporin in ulcerative colitis: a five-year experience. *Am J Gastroenterol* 1999;94:1587-1592.

Conway PL. Microbial ecology of the human large intestine. In: *Human colonic bacteria: Role in Nutrition, Physiology and Pathology*: Ginson GR, Macfarlane GT, eds. Boca Raton: CRC Press, 1995:1-24.

Cope GF, Heatley RV, Kelleher JK. Smoking and colonic mucus in ulcerative colitis. *BMJ* 1986;293:481.

Corrao G, Tragnone A, Caprilli R, Trallori G, Papi C, Andreoli A, Paolo M, Riegler G, Rigo G, Ferrau O, Mansi C, Ingrosso M, Valp D. Risk of inflammatory bowel disease attributable to smoking, oral contraception and breastfeeding in Italy; a nationwide case-control study. *Int J Epidemiol* 1998;27:397-404.

Coulie B, Camilleri M, Bharucha AE, Sandborn WJ, Burton D. Colonic motility in chronic ulcerative proctosigmoiditis and the effects of nicotine on colonic motility in patients and healthy subjects. *Aliment Pharmacol Ther* 2001;15:653-663.

d'Albasio G, Pacini F, Camarri E, Messori A, Trallori G, Bonanomi AG, Bardazzi G, Milla M, Ferrero S, Biagini M, Quaranta S, Amorosi A. Combined therapy with 5-aminosalicylic acid tablets and enemas for maintaining remission in ulcerative colitis: a randomized double-blind study. *Am J Gastroenterol* 1997;92:1143-1147.

Darfeuille-Michaud A, Neut C, Barnich N, Lederman E, Di-Martino P, Desreumaux P, Gambiez L, Joly B, Cortot A, Colombel JF. Presence of adherent *Escherichia coli* strains in ileal mucosa of patients with Crohn's disease. *Gastroenterology* 1998;115: 1405-1413.

Das KM, Dasgupta A, Mandal A, Geng X. Autoimmunity to cytoskeletal protein tropomyosin: A new clue to the pathogenetic mechanism for ulcerative colitis. *J Immunol* 1993;150: 2487-2493.

Das KM, Vecchi M, Sakamaki S. A shared and unique epitope on human colon, skin and biliary epithelium detected by a monoclonal antibody. *Gastroenterology* 1990;98: 464-469.

Dayton MT, Larsen KR, Christiansen DD. Similar functional results and complications after ileal pouch-anal anastomosis in patients with indeterminate vs ulcerative colitis. *Arch Surg* 2002;137:690-694.

Di Febo G, Miglioli M, Lauri A, Biasco G, Paganelli GM, Poggioli G, Gozzetti G, Barbara L. Endoscopic assessment of acute inflammation of the reservoir after restorative ileoanal anastomosis. *Gastrointest Endosc* 1990;36:6-9.

de Silva HJ, Ireland A, Kettlewell M, Mortensen NJ, Jewell DP. Short chain fatty acid irrigation in severe pouchitis (letter). *N Engl J Med* 1989;321:1416.

de Silva HJ, Millard PR, Kettlewell M, Mortensen NJ, Prince C, Jewell DP. Mucosal characteristics of pelvic ileal pouches. *Gut* 1991;32:1160-1165 (b).

de Silva HJ, Millard PR, Snoper N, Kettlewell, Mortensen N, Jewell DP. Effect of the faecal stream and stasis on the ileal pouch mucosa. *Gut* 1991; 32: 1166-9 (a).

de Vlam K, Mielants H, Cuvelier C, De Keyser F, Veys EM, De Vos M. Spondyloarthropathy is underestimated in inflammatory bowel disease: prevalence and HLA association. *J Rheumatol* 2000;27:2860-2865.

Dekker-Saeys BJ, Meuwissen SG, Van Den Berg-Loonen EM, De Haas WH, Meijers KA. Clinical characteristics and results of histocompatibility typing (HLA B27) in 50 patients with both ankylosing spondylitis and inflammatory bowel disease. *Ann Rheum Dis* 1978;37:36-41.

Duerr RH, Neigut DA. Molecularly defined HLA-DR2 alleles in ulcerative colitis and an antineutrophil cytoplasmic antibody positive subgroup. *Gastroenterology* 1995;108:423-427.

Duerr RH, Targan SR, Landers CJ, LaRusso NF, Lindsay KL, Wiesner RH, Shanahan F. Neutrophil cytoplasmic antibodies: a link between primary sclerosing cholangitis and ulcerative colitis. *Gastroenterology*. 1991;100:1385-1391.

Edward F, Truelove S. The course and prognosis of ulcerative colitis.I. *Gut* 1963;4:299-315.

Edward F, Truelove S. The course and prognosis of ulcerative colitis. III. Complications. *Gut* 1964;5:1-22.

Elson CO, Sartor RB, Tennyson G, Riddel R. Experimental models of inflammatory bowel disease. *Gastroenterology* 1995;109:1344-1367.

Esteve M, Mallolas J, Klaassen J, Abadlacruz A, Conzalezhuix F, Cabre E, Fernandezbanarez F, Bertran X, Condom E, Martiraque J, Gassull MA. Antineutrophil cytoplasmic antibodies in sera from colectomised ulcerative colitis patients and its relation to the presence of pouchitis. *Gut* 1996;38:894-898.

Fazio VW, Ziv Y, Church JM, Oakley JR, Lavery IC, Milsom JW, Schroeder TK. Ileal pouch-anal anastomoses complications and function in 1005 patients. *Ann Surg* 1995;222:120-127.

Fernandez-Banares F, Abad-Lacruz A, Xiol X, Gine JJ, Dolz C, Cabre E, Esteve M, Gonzalez-Huix F, Gassull MA. Vitamin status in patients with inflammatory bowel disease. *Am J Gastroenterol* 1989; 84:744-748.

Ferretti M, Casini-Raggi V, Pizarro TT, Eisenberg SP, Nast CC, Cominelli F. Neutralization of endogenous IL-1 receptor antagonist exacerbates and prolongs inflammation in rabbit immune colitis. *J Clin Invest* 1994;94:449-453.

Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* 1998;115:182-205.

Fischer JE, Nussbaum MS, Martin LW, Warner BW, Flesch L, Staneck JL, Niemer E, Bjornson HS, Thompson R, McFadden DW. The pull-through procedure: technical factors in influencing outcome, with emphasis on pouchitis. *Surgery* 1993;114:828-835.

Fleshman JW, Cohen Z, McLeod RS, Stern H, Blair J. The ileal reservoir and ileoanal anastomosis procedure. Factors affecting technical and functional outcome. *Dis Colon Rectum* 1988;31:10-16.

Fleshner PR, Vasilias EA, Kam LY, Fleshner NE, Gaiennie J, Abreu-Martin MT, Targan SR. High level perinuclear antineutrophil cytoplasmic antibody (pANCA) in ulcerative colitis patients before colectomy predicts the development of chronic pouchitis after ileal pouch anal anastomosis. *Gut* 2001; 49: 671-677.

Fuller R. Probiotics in man and animal. *J Appl Bacteriol* 1989;66:365-378.

Futami S, Aoyama N, Honsako Y, Tamura T, Morimoto S, Nakashima T, Ohmoto A, Okano H, Miyamoto M, Inaba H. HLA-DRB1\*1502 allele, subtype of DR15, is associated with susceptibility to ulcerative colitis and its progression. *Dig Dis Sci* 1995;40:814-818.

Galandiuk S, Scott NA, Dozois RR, Kelly KA, Ilstrup DM, Beart RW Jr, Wolff BG, Pemberton JH, Nivatvongs S, Devine RM. Ileal pouch-anal anastomosis: Reoperation for pouch-related complications. *Ann Surg* 1990;212:446-454.

Geerling BJ, Dagnelie PC, Badart-Smook A, Russel MG, Stockbrugger RW, Brummer RJM. Diet as a risk factor for the development of ulcerative colitis. *Am J Gastroenterol* 2000;95:1008-1013.

Geerling BJ, Stockbrugger RW, Brummer RJ. Nutrition and inflammatory bowel disease: An update. *Scand J Gastroenterol Suppl* 1999; 230: 95–105.

Giaffer MH, Holdsworth CD, Duerden BI. Virulence properties of *Escherichia coli* strains isolated from patients with inflammatory bowel disease. *Gut* 1992;33: 646-650.

Gionchetti P, Campieri M, Belluzzi A, Bertinelli E, Ferretti M, Brignola C, Poggioli G, Miglioli M, Barbara L. Mucosal concentrations of interleukin-1 $\beta$ , interleukin-6, interleukin-8, and tumor necrosis factor- $\alpha$  in pelvic ileal pouches. *Dig Dis Sci* 1994;39:1525-1531.

Gionchetti P, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G, Poggioli G, Miglioli M, Campieri M. Oral bacteriotherapy as a maintenance treatment in patients with chronic pouchitis: A double-blind placebo-controlled trial. *Gastroenterology* 2000;119:305-309 (a).

Gionchetti P, Rizzello F, Venturi A, Ugolini F, Rossi M, Brigidi P, Johansson R, Ferrieri A, Poggioli G, Campieri M. Antibiotic combination therapy in patients with chronic, treatment-resistant pouchitis. *Aliment Pharmacol Ther* 1999;13:713-718.

Gionchetti P, Rizzello F, Venturi A, Helwig U, Amadini C, Lammers KM, Ugolini F, Poggioli G, Campieri M. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind placebo controlled trial. *Gastroenterology* 2000;118:A190 (b).

Glassman M, Newman LJ, Berezin S, Stockbrugger R, Brummer R, Gryboski J. Cow's milk protein sensitivity during infancy in patients with inflammatory bowel disease. *Am J Gastroenterol* 1990;85:838-840.

Go PM, van Diejen-Visser MP, Davies BI, Lens J, Brombacher PJ. Microbial flora and bile acid metabolism in patients with an ileal reservoir. *Scand J Gastroenterol* 1988;23:229-236.

Goudet P, Dozois RR, Kelly KA, Ilstrup DM, Phillips SF. Characteristics and evolution of extraintestinal manifestations associated with ulcerative colitis after proctocolectomy. *Digestive Surgery* 2001;18:51-55.

Greenstein AJ, Janowitz HD, Sachar DB. The extra-intestinal complications of Crohn's disease and ulcerative colitis: A study of 700 patients. *Medicine (Baltimore)* 1976;55:401-412.

Grundy SM, Ahrens EH Jr, Miettinen TA. Quantitative isolation and gas-liquid chromatographic analysis of total fecal bile acids. *J Lipid Res* 1965;6:397-410.

Gullberg K, Ståhlberg D, Liljeqvist L, Tribukait B, Reinholt FP, Veress B, Löfberg R. Neoplastic transformation of the pelvic pouch mucosa in patients with ulcerative colitis. *Gastroenterology* 1997; 112:1487-1492.

Hakala K, Vuoristo M, Luukkonen P, Jarvinen HJ, Miettinen TA. Impaired absorption of cholesterol and bile acids in patients with an ileoanal anastomosis. *Gut* 1997;41:71-77(a).

Hakala K, Luukkonen P, Vuoristo M, Jarvinen H, Miettinen TA. Cholesterol metabolism and non-cholesterol sterols in patients with ileal pouch anastomosis. *J Hepatol* 1997; 26:1306-1312(b).

Hanauer S. Medical therapy for ulcerative colitis. In: Kirsner JB ed. *Inflammatory Bowel Disease* 5<sup>th</sup> ed. WB Saunders company, 2000;529-556.

Harig JM, Soergel KH, Komorowski RA, Wood CM. Treatment of diversion colitis with short chain fatty acid irrigation. *N Engl J Med* 1989;320:23-28.

Hawthorne AB, Logan RF, Hawkey CJ, Foster PN, Axon AT, Swarbrick ET, Scott BB, Lennard-Jones JE. Randomised controlled trial of azathioprine withdrawal in ulcerative colitis. *BMJ* 1992;305:20-22.

Heuschen UA, Autschbach F, Allemeyer EH, Zöllinger AM, Heuschen G, Uehlein T, Herfarth Ch, Stern J. Long-term follow-up after ileoanal pouch procedure. *Dis Colon Rectum* 2001;44:487-499 (a).

Heuschen UA, Heuschen G, Autschbach F, Allemeyer EH, Herfarth C. Adenocarcinoma in the ileal pouch: late risk of cancer after restorative proctocolectomy. *Int J Colorectal Dis* 2001;16:126-130 (b).

Holma R, Salmenperä P, Lohi J, Vapaatalo H, Korpela R. Effects of *Lactobacillus rhamnosus* GG and *Lactobacillus reuteri* R2LC on acetic acid-induced colitis in rats. *Scand J Gastroenterol* 2001;36: 630-635.

Hurst R, Molinari M, Philip Chung TP, Rubin M, Michelassi F. Prospective study of the incidence, timing, and treatment of pouchitis in 104 consecutive patients after restorative proctocolectomy. *Arch Surg* 1996;131:497-502.

Hylander E, Rannem T, Hegnhøj J, Kirkegaard P, Thale M, Jarnum S. Absorption studies after ileal J-pouch anastomosis for ulcerative colitis. A prospective study. *Scand J Gastroenterol* 1991;6:65-72.

Ikeda I, Kasajima T, Ishiyama S, Shimojo T, Takeo Y, Nishikawa T, Kameoka S, Hiroe M, Mitsunaga A. Distribution of inducible nitric oxide synthase in ulcerative colitis. *Am J Gastroenterol* 1997;92:1339-1341.

Ishiguro Y. Mucosal proinflammatory cytokine production correlates with endoscopic activity of ulcerative colitis. *J Gastroenterol* 1999;34:66-74.

Ito M, Rimura M. Influence of lactose on faecal microflora in lactose maldigesters. *Microb Ecol Health Dis* 1993; 6: 73-6.

Jarnerot G, Anderson M, Franzen L. Laparoscopic appendectomy in patients with refractory ulcerative colitis. *Gastroenterology* 2001;120:1562-1563.

Jewell DP. Ulcerative colitis. In: Feldman M, Scharschmidt BF, Sleisenger M. *Gastrointestinal and Liver disease*. 6<sup>th</sup> ed. W.B.Saunders Company 1998.p.1735-1761.

Jimmo B, Hyman NH. Is ileal pouch-anal anastomosis really the procedure of choice for patients with ulcerative colitis? *Dis Colon Rectum* 1998;4:41-45.

Joelsson M, Andersson M, Bark T, Gullberg K, Hallgren T, Jiborn H, Magnusson I, Raab Y, Sjødahl R, Ojerskog B, Oresland T. Allopurinol as prophylaxis against pouchitis following ileal pouch-anal anastomosis for ulcerative colitis. A randomized placebo-controlled double-blind study. *Scand J Gastroenterol*. 2001;36:1179-84.

Johnson E, Carlsen E, Nazir M, Nygaard K. Morbidity and functional outcome after restorative proctocolectomy for ulcerative colitis. *Eur J Surg* 2001;167:40-45.

Jousimies-Somer HR, Summanen PH, Finegold SM. *Bacteroides*, *porphyromonas*, *prevotella*, *fucobacterium* and other anaerobic gram-negative rods and cocci. In: Murray PR, Baron EJ, editors. *Manual of clinical microbiology*. 7<sup>th</sup> ed. Washington DC:ASM press;1999.p.690-713.

Kanai T, Watanabe M, Okazawa A, Sato T, Yamazaki M, Okamoto S, Ishii H, Totsuka T, Iiyama R, Okamoto R, Ikeda M, Kurimoto M, Takeda K, Akira S, Hibi T. Macrophage-derived IL-18-mediated intestinal inflammation in the murine model of Crohn's disease. *Gastroenterology*. 2001;12:875-88.

Kartheuser AH, Parc R, Penna CP, Turet E, Frileux P, Hannoun L, Nordlinger B, Loygue J. Ileal pouch-anal anastomosis as the first choice operation in patients with familial adenomatous polyposis: a ten-year experience. *Surgery* 1996;119:615-623.

Keränen U, Järvinen H, Kärkkäinen P, Kiviluoto T, Kivilaakso E, Soinila S. Substance P- an underlying factor for pouchitis? Prospective study of substance P- and vasoactive intestinal polypeptide-immunoreactive innervation and mast cells. *Dig Dis Sci* 1996 41:1665-1671.

Keränen U, Luukkonen P, Järvinen H. Functional results after restorative proctocolectomy complicated by pouchitis. *Dis Colon Rectum* 1997;40:764-769.

Kimura H, Hokari R, Miura S, Shigematsu T, Hirokawa M, Akiba Y, Kurose I, Higuchi H, Fujimori H, Tsuzuki Y, Serizawa H, Ishii H. Increased expression of an inducible isoform of nitric oxide synthase and the formation of peroxynitrite in colonic mucosa in patients with active ulcerative colitis. *Gut* 1998;42:180-187.

Kmiot WA, Hesselwood SR, Smith N, Thompson H, Harding LK, Keighley MRB. Evaluation of the inflammatory infiltrate in pouchitis with <sup>111</sup>indium-labeled granulocytes. *Gastroenterology* 1993;104:981-989.

Kock NG, Brevinge H, Philipson BM, Ojerskog B. Continent ileostomy. The present technique and long term results. *Ann Chir Gynaecol.* 1986;75(2):63-70.

Kohler LW, Pemberton JH, Zinsmeister AR, Kelly KA. Quality of life after proctocolectomy. A comparison of Brooke ileostomy, Kock pouch and ileal pouch-anal anastomosis. *Gastroenterology* 1991;101: 679-681.

Kühbacher T, Schreiber S, Runkel N. Pouchitis: pathophysiology and treatment. *Int J Colorectal Dis* 1998;13:196-207.

Langholz E, Munkholm P, Davidsen M, Binder V. Course of ulcerative colitis: analysis of changes in disease activity over years. *Gastroenterology* 1994;107:3-11.

Leong AP, Londono-Schimmer EE, Phillips RK. Life-table analysis of stomal complications following ileostomy. *Br J Surg* 1994;81: 727-729.

Lepistö A, Luukkonen P, Järvinen HJ. Cumulative failure rate of ileal pouch-anal anastomosis and quality of life after failure. *Dis Colon Rectum.* 2002;45:1289-94.

Lepistö AH, Järvinen HJ. Durability of Kock continent ileostomy. *Dis Colon Rectum.* 2003;46:925-928.

Lichtiger S, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, Michelassi F, Hanauer S. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J Med* 1994;330:1841-1845.

Lin CL, Moniz C, Chambers TJ, Chow JW. Colitis causes bone loss in rats through suppression of bone formation. *Gastroenterology* 1996;111:1263-1271.

Lohmuller JL, Pemberton JH, Dozois RR, Ilstrup D, van Heerden J. Pouchitis and extraintestinal manifestations of inflammatory bowel disease after ileal pouch-anal anastomosis. *Ann Surg* 1990;211:622-629.

Luukkonen P, Järvinen H, Tanskanen M, Kahri A. Pouchitis- recurrence of the inflammatory bowel disease? *Gut* 1994;35:234-46.

Luukkonen P, Valtonen V, Sivonen A, Sipponen P, Järvinen H. Fecal bacteriology and reservoir ileitis in patients operated on for ulcerative colitis. *Dis Colon Rectum* 1988;31:864-867.

M'Koma AE, Lindquist K, Liljequist L. Biochemical laboratory data in patients before and after restorative proctocolectomy. A study on 83 patients with a follow-up of 36 months. *Ann Chir* 1994; 48: 525 – 534.

M'Koma AE. Follow-up results of hematology data before and after restorative proctocolectomy. Clinical outcome. *Dis Colon Rectum* 1994; 37: 932 –937.

Madden M, McIntyre A, Nicholls RJ. Double-blind cross-over trial of metronidazole versus placebo in chronic unremitting pouchitis. *Dig Dis Sci* 1994;39:1193-1196.

Madden MV, Farthing MJG, Nicholls RJ. Inflammation in ileal reservoirs: 'pouchitis'. *Gut* 1990;31:247-249.

Madretsma S, Wolters LM, van Dijk JP, Tak CJ, Feyerabend C, Wilson JH, Zijlstra FJ. In-vivo effect of nicotine on cytokine production by human non-adherent mononuclear cells. *Eur J Gastroenterol Hepatol* 1996;8:1017-1020.

Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. Lactobacillus species prevent colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 1999;116:1107-1114.

Mahadevan U, Sandborn WJ. Diagnosis and management of pouchitis. *Gastroenterology* 2003;124:1636-1650.

Marteau PR, de Vrese M, Cellier CJ, Schrezenmeir J. Protection from gastrointestinal diseases with the use of probiotics. *Am J Clin Nutr* 2001;73:430S-436S.

McCallum DI, Kinmont PD. Dermatological manifestations of Crohn's disease. *Br J Dermatol* 1968;80:1-8.

Meagher AP, Farouk R, Dozois RR, Kelly KA, Pemberton JH. J ileal pouch-anal anastomosis for chronic ulcerative colitis: complications and long-term outcome in 1310 patients. *Br J Surg* 1998;85:800-803.

Merret MN, Mortensen N, Kettlewell M, Jewell DO. Smoking may prevent pouchitis in patients with restorative proctocolectomy for ulcerative colitis. *Gut* 1996;38:362-364.

Meucci G, Vecchi M, Astegiano M, Beretta L, Cesari P, Dizioli P, Ferraris L, Panelli M, Prada A, Sostegni R, de Francis R. The natural history of ulcerative proctitis: a multicenter, retrospective study. *Am J Gastroenterol* 2000;95:469-473.

Miglioli M, Barbara L, Di Febo G, Gozzetti G, Lauri A, Paganelli GM, Poggioli G, Santucci R. Topical administration of 5-aminosalicylic acid: a therapeutic proposal for the treatment of pouchitis. *N Engl J Med* 1992;320:257.

Miller LG, Goldstein G, Murphy M, Ginns LC. Reversible alterations in immunoregulatory T cells in smoking. *Chest* 1982;5:526-529.

Mimura T, Rizzello F, Helwig U, Poggioli G, Schreiber S, Talbot IC, Nicholls RJ, Gionchetti P, Campieri M, Kamm MA. Four-week open-label trial of metronidazole and ciprofloxacin for the treatment of recurrent or refractory pouchitis. *Aliment Pharmacol Ther* 2002;16:909-917.

Mir-Madjlessi SH, Taylor JS, Farmer RG. Clinical course and evolution of erythema nodosum and pyoderma gangrenosum in chronic ulcerative colitis. A study of 42 patients. *Am J Gastroenterol* 1985;80:615-620.

Mizoguchi A, Mizoguchi E, Chiba C, Bhan AK. Role of appendix in the development of inflammatory bowel disease in TCR-alpha mutant mice. *J Exp Med* 1996;184:707-715.

Monsen U, Sorstad J, Hellers G, Johansson C. Extracolonic diagnosis in ulcerative colitis: An epidemiological study. *Am J Gastroenterol* 1990;85:711-716.

Moskowitz RL, Shephard NA, Nicholls RJ. An assessment of inflammation in the reservoir after restorative proctocolectomy with ileal reservoir. *Int J Colorectal Dis* 1986;1:67-174.

Motley RJ, Rhodes J, Ford GA, Wilkinson SP, Chesner IM, Asquith P, Hellier MD, Mayberry JF. Time relationships between cessation of smoking and onset of ulcerative colitis. *Digestion* 1987;37:125-127.

Naganuma M, Iizuka B, Torii A, Ogihara T, Kawamura Y, Ichinose M, Kojima Y, Hibi T; Tokyo Gut Club. Appendectomy protects against the development of ulcerative colitis and reduces its recurrence. Results of a multicenter case-controlled study in Japan. *Am J Gastroenterol* 2001;96:1123-1126.

Naparstek Y, Plotz PH. The role of autoantibodies in autoimmune disease. *Annu Rev Immunol* 1993; 11: 79-104.

Nasmyth DG, Godwin PG, Dixon MF, Williams NS, Johnston D. Ileal ecology after pouch-anal anastomosis or ileostomy. A study of mucosal morphology, fecal bacteriology, fecal volatile fatty acids and their interrelationship. *Gastroenterology* 1989;96:817-24.

Natori H, Utsunomiya J, Yamamura T, Benno Y, Uchida K. Fecal and stomal bile acid composition after ileostomy or ileoanal anastomosis in patients with chronic ulcerative colitis and adenomatosis coli. *Gastroenterology* 1992;102:1278-88.

Nicholls RJ. Review article: ulcerative colitis-surgical indications and treatment. *Aliment Pharmacol Ther* 2002;16(Suppl.4)25-28.

Nicklin M, Weith A, Duff GW. A physical map of the region encompassing the human interleukin-1 alpha, interleukin-1 beta and interleukin-1 receptor antagonist genes. *Genomics* 1994;19:382-384.

Nygaard K, Bergan T, Bjornekleit A, Hoverstad T, Lassen J, Aase S. Topical metronidazole treatment in pouchitis. *Scand J Gastroenterol* 1994;29:462-467.

O'Connell PR, Rankin DR, Weiland LH, Kelly KA. Enteric bacteriology, absorption, morphology and emptying after ileal pouch-anal anastomosis. *Br J Surg* 1986;73:909-914.

Olsen KO, Joelson M, Laurberg S, Oresland T. Fertility after ileal pouch-anal anastomosis in women with ulcerative colitis. *Br J Surg* 1999;86:493-495.

Olsson R, Danielsson A, Jarnerot G, Lindstrom E, Loof L, Rolny P, Ryden BO, Tysk C, Wallerstedt S. Prevalence of primary sclerosing cholangitis in patients with ulcerative colitis. *Gastroenterology* 1991;100:1319-1323.

Onderdonk AB, Dvorak AM, Cisneros RL, McLeod RS, Antionoli D, Silen W, Blair JE, Monahan-Earley RA, Cullen J, Cohen Z. Microbiologic assessment of tissue biopsy samples from ileal pouch patients. *J Clin Microbiol* 1992;30:312-7.

Orchard TR, Thiyagaraja S, Welsh KI, Wordsworth BP, Hill Gaston JS, Jewell DP. Clinical phenotype is related to HLA genotype in the peripheral arthropathies of inflammatory bowel disease. *Gastroenterology* 2000;118: 274-278.

Orchard TR, Wordsworth BP, Jewell DP. Peripheral arthropathies in inflammatory bowel disease: Their articular distribution and natural history. *Gut* 1998; 42:387-391.

Orholm M, Binder V, Sorensen TI, Rasmussen LP, Kyvik KO. Concordance of inflammatory bowel disease among Danish twins: results of a nationwide study. *Scand J Gastroenterol* 2000;35:1075-1781.

Orholm M, Munkholm P, Langholz E, Nielsen OH, Sorensen IA, Binder V. Familial occurrence of inflammatory bowel disease. *N Engl J Med* 1991;324:84-88.

Palm O, Moum B, Jahnsen J, Gran JT. The prevalence and incidence of peripheral arthritis in patients with inflammatory bowel disease, a prospective population-based study (the IBSEN study). *Rheumatology* 2001;40:1256-1261.

Papo M, Quer JC, Pastor RM, Garcia-Pardo G, Prats E, Mirapeix E, Rodriguez R, Richart C. Antineutrophil cytoplasmic antibodies in relatives of patients with inflammatory bowel disease. *Am J Gastroenterol* 1996;91:1512-1515.

Park AG, Nicholls RJ. Proctocolectomy without ileostomy for ulcerative colitis. *Br Med J* 1978;2:85-88.

Patel RT, Bain I, Youngs D, Keighley MR. Cytokine production in pouchitis is smimilar to that in ulcerative colitis. *Dis Colon Rectum* 1995;38:831-837.

Penna C, Dozois R, Tremaine W, Sandborn W, LaRusso N, Schleck C, Ilstrup D. Pouchitis after ileal pouch-anal anastomosis for ulcerative colitis occurs with increased frequency in patients with associated primary sclerosing cholangitis. *Gut* 1996;38:234-239.

Pera A, Bellando P, Caldera D, Ponti V, Astegiano M, Barletti C, David E, Arrigoni A, Rocca G, Verme G. Colonoscopy in inflammatory bowel disease. Diagnostic accuracy and proposal of an endoscopic score. *Gastroenterology*. 1987;92:181-185.

Perbeck L, Lindquist K, Liljequist L. The mucosal bloodflow in pelvic pouches is man: a methodologic study of fluorescein flowmetry. *Dis Colon Rectum* 1985;28:931-936.

Philipson B, Brandberg A, Jagenburg R, Kock NG, Lager I, Ahren C. Mucosal morphology, bacteriology and absorption in intra-abdominal ileostomy reservoir. *Scand J Gastroenterol* 1975;10:145-153.

Phillips J, Muir JG, Birkett A, Lu ZX, Jones GP, O'Dea K, Young GP. Effect of resistant starch on fecal bulk and fermentation-dependent events in humans. *Am J Clin Nutr* 1995;62:121-30.

Podolsky DK, Isselbacher KJ. Glykoprotein composition of colonic mucosa: spesific alterations in ulcerative colitis. *Gastroenterology* 1984;87:991-98.

Pollak RD, Karmeli F, Eliakim R, Ackerman Z, Tabb K, Rachmilewitz D. Femoral neck osteopenia in patients with inflammatory bowel disease. *Am J Gastroenterol* 1998;93:1483-90.

Probert CS, Jayanthi V, Pinder D, Wicks AC, Mayberry JF. Epidemiological study of ulcerative colitis in Indian migrants and the indigenous population of Leicestershire. *Gut* 1992;33:687-693.

Prudhomme M, Dozois RR, Godlewski G, Mathison S, Fabbro-Peray P. Anal canal strictures after ileal pouch-anal anastomosis. *Dis Colon Rectum* 2003;46:20-23.

Rachmilewitz D, Eliakim R, Ackerman Z, Karmeli F. Direct determination of colonic nitric oxide level- a sensitive marker of disease activity in ulcerative colitis. *Am J Gastroenterol* 1998;93:409-412.

Rachmilewitz D, Karmeli F, Takabayashi K, Hayashi T, Leider-Trejo L, Lee J, Leoni LM, Raz E. Immunostimulatory DNA ameliorates experimental and spontaneous murine colitis. *Gastroenterology*. 2002;122:1428-41.

Raj D, Lichtenstein DR. Hepatobiliary manifestations of inflammatory bowel disease. *Gastroenterol Clin N Am* 1999;28:491-513.

Rath HC, Schultz M, Freitag R, Dieleman LA, Li F, Linde HJ, Scholmerich J, Sartor RB. Different subsets of enteric bacteria induce and perpetuate experimental colitis in rats and mice. *Infect Immun* 2001;69:2277-2285.

Reif S, Klein I, Lubin F, Farbstein M, Hallak A, Gilat T. Pre-illness dietary factors in inflammatory bowel disease. *Gut* 1997;40:754-60.

Reif S, Lavy A, Keter D, Broide D, Niv Y, Halak A, Ron Y, Eliakim R, Odes S, Patz J, Fich A, Villa Y, Arber N, Gilat T. Appendectomy is more frequent but not a risk factor in Crohn's disease while being protective in ulcerative colitis: a comparison of surgical procedures in inflammatory bowel disease. *Am J Gastroenterol* 2001;96:829-832.

Rhodes JM. Colonic mucus and mucosal glycoproteins: the key to colitis and cancer? *Gut* 1989;30:1660-1666.

Romanos J, Samarasekera DN, Stebbing JF, Jewell DP, Kettlewell MGW, Mortensen NJ. Outcome of 200 restorative proctocolectomy operations: the John Radcliffe Hospital experience. *Br J Surg* 1997;84:814-818.

Roussomoustakaki M, Satsangi J, Welsh K, Louis E, Fanning G, Targan S, Landers C, Jewell DP. Genetic markers may predict disease behavior in patients with ulcerative colitis. *Gastroenterology* 1997;112:1845-1853.

Ruseler-van Emden JGH, Schouten WR, van Lieshout LMC. Pouchitis: result of microbial imbalance? *Gut* 1994;35:658-64.

Russel MG, Dorant E, Brummer RJ, van de Kruijs MA, Muris JW, Bergers JM, Goedhard J, Stockbrugger RW. Appendectomy and the risk of developing ulcerative colitis or Crohn's disease: results of a large case-control study. *Gastroenterology* 1997;113:377-382.

Rutgeerts P, D'Haens G, Hiele M, Geboes K, Vantrappen G. Appendectomy protects against ulcerative colitis. *Gastroenterology* 1994;106:1251-1253.

Safdi M, DeMicco M, Sninsky C, Banks P, Wruble L, Deren J, Koval G, Nichols T, Targan S, Fleishman C, Wiita B. A double-blind comparison of oral versus rectal mesalamine versus combination therapy in the treatment of distal ulcerative colitis. *Am J Gastroenterol* 1997;92:1867-1871.

Sagar PM, Pemberton JH. Ileo-anal pouch function and dysfunction. *Dig Dis Sci* 1997;15:172-188.

Sagar PM, Taylor BA, Godwin P, Holdsworth PJ, Johnston D, Lewis W, Miller A, Quirke P, Williamson M. Acute pouchitis and deficiencies of fuel. *Dis Colon Rectum* 1995;38:488-493.

Samarasekera DN, Stebbing JF, Kettlewell MG, Jewell DP, Mortensen NJ. Outcome of restorative proctocolectomy with ileal reservoir for ulcerative colitis: comparison of distal colitis with more proximal disease. *Gut* 1996;38:574-577.

Sambuelli A, Boerr L, Negreira S, et al. Budesonide enemas versus oral metronidazole in pouchitis. A double-blind double-dummy controlled trial. *Gut* 2000;47(S3): A249.

Sandborn WJ, Landers CJ, Tremaine WJ, Targan SR. Antineutrophil cytoplasmic antibody correlates with chronic pouchitis after ileal pouch-anal anastomosis. *Gastroenterology* 1995;90: 740-746.

Sandborn WJ, Landers CJ, Tremaine WJ, Targan SR. Association of antineutrophil cytoplasmic antibodies with resistance to treatment of left-sided ulcerative colitis: results of a pilot study. *Mayo Clin Proc* 1996;71: 431-436.

Sandborn WJ, Tremaine WJ, Batts KP, Pemberton JH, Phillips SF. Pouchitis following ileal pouch-anal anastomosis: A pouchitis disease activity index. *Mayo Clin Proc* 1994; 69: 409-415 (b).

Sandborn WJ, Tremaine WJ, Batts KP, Pemberton JH, Rossi SS, Hofmann AF, Gores GJ, Phillips SF. Fecal bile acids, short-chain fatty acids, and bacteria after ileal pouch-anal anastomosis do not differ in patients with pouchitis. *Dig Dis Sci* 1995;40:1474-1483.

Sandborn WJ. Pouchitis following ileal pouch anal anastomosis: definition, pathogenesis, and treatment. *Gastroenterology* 1994;107:1856-1860(a)

Santavirta J, Harmoinen A, Karvonen AL, Matikainen M. Water and electrolyte balance after ileoanal anastomosis. *Dis Colon Rectum* 1991;34:115-118 (b).

Santavirta J, Mattila J, Kokki M, Pöyhönen L, Matikainen M. Absorption of bile acids after ileoanal anastomosis. *Ann Chir Gynaecol* 1990; 79:134 -138.

Santavirta J, Mattila J, Kokki M, Matikainen M. Mucosal morphology and faecal bacteriology after ileoanal anastomosis. *Int J Colorect Dis* 1991;6:38-41(a).

Sartor R. Microbial factors in the pathogenesis of Crohn's disease, ulcerative colitis, and experimental intestinal inflammation. In: Kirsner JB, ed. *Inflammatory bowel disease*, 5th edn. Philadelphia: WB Saunders, 2000:153-178.

Sartor RB. Cytokines in intestinal inflammation: Pathophysiological and clinical considerations. *Gastroenterology* 1994;106: 533-539.

Satsangi J, Welsh KI, Bunce M, Julier C, Farrant JM, Bell JI, Jewell DP. Contribution of genes of the major histocompatibility complex to susceptibility and disease phenotype in inflammation bowel disease. *Lancet* 1996; 347:1212-1217 (a).

Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP. Two-stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nat Genet* 1996;14:199-202 (b).

Savage DC. Microbial ecology of the gastrointestinal tract. *Annu Rev Microbiol* 1977;31:107-133.

Saxon A, Shanahan F, Landers C, Ganz T, Targan S. A distinct subset of antineutrophil cytoplasmic antibodies is associated with inflammatory bowel disease. *J Allergy Clin Immunol* 1990;86:202-210.

Scharla SH, Minne HW, Lempert UG, Leidig G, Hauber M, Raedsch R, Ziegler R. Bone mineral density and calcium regulating hormones in patients with inflammatory bowel disease (Crohn's disease and ulcerative colitis). *Exp Clin Endocrinol* 1994;102:44-9.

Schley PD, Field CJ. The immune-enhancing effects of dietary fibres and prebiotics. *Br J Nutr* 2002;87:S221-S230.

Schmidt CM, Lazenby AJ, Hendrickson RJ, Sitzmann JV. Preoperative terminal ileal and colonic resection histopathology predicts risk of pouchitis in patients after ileoanal pull-through procedure. *Ann Surg* 1998;227:654-665.

Schultz C, Van Den Berg FM, Ten Kate FW, Tytgat GN, Dankert J. The intestinal mucus layer from patients with inflammatory bowel disease harbors high numbers of bacteria compared with controls. *Gastroenterology* 1999;117:1089-1097.

Scott AD, Phillips RK. Ileitis and pouchitis after colectomy for ulcerative colitis. *Br J Surg* 1989;76:668-669.

Seibold F, Brandwein S, Simpson S, Terhorst C, Elson CO. pANCA represents a cross-reactivity to enteric bacterial antigens. *J Clin Immunol* 1998;18:153-160.

Setti Carraro PG, Talbot IC, Nicholls JR. Patterns of distribution of endoscopic and histological changes in the ileal reservoir after restorative proctocolectomy for ulcerative colitis. A long-term follow-up study. *Int J Colorectal Dis.* 1998;13:103-107.

Shen B, Achkar JP, Lashner A, Ormsby AH, Remzi FH, Bevins CL, Brzezinski A, Petras RE, Fazio VW. Endoscopic and histologic evaluation together with symptom assessment are required to diagnose pouchitis. *Gastroenterology* 2001;121:261-267(a).

Shen B, Achkar JP, Lashner BA, Ormsby AH, Brzezinski A, Soffer EE, Remzi FH, Bevins CL, Fazio VW. Irritable pouch syndrome: a new category of diagnosis for symptomatic patients with ileal pouch-anal anastomosis. *Am J Gastroenterol* 2002;97: 927-927.

Shen B, Achkar JP, Lashner BA, Ormsby AH, Remzi FH, Brzezinski A, Bevins CL, Bambrick ML, Seidner DL, Fazio VW. A randomized clinical trial of ciprofloxacin and metronidazole to treat acute pouchitis. *Inflamm Bowel Dis* 2001;7:301-5 (b).

Shepherd NA, Healey CJ, Warren BF, Richman PI, Thomson WH, Wilkinson SP. Distribution of mucosal pathology and an assessment of colonic phenotypic change in the pelvic ileal reservoir. *Gut* 1993;34:101-105.

Shepherd NA, Jass JR, Duval I, Moskowitz RL, Nicholls RJ, Morson BC. Restorative proctocolectomy with ileal reservoir: pathological and histochemical study of mucosal biopsy specimens. *J Clin Pathol* 1987; 40: 601 –607.

Shepherd NA. The pelvic ileal reservoir: apocalypse later? Patients need monitoring for the long term effects of reservoir construction. *BMJ* 1990;301: 886-887.

Sher ME, Bank S, Greenberg R, Sardinha TC, Weissman S, Bailey B, Gilliland R, Wexner SD. The influence of cigarette smoking on cytokine levels in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 1999;5:73-78.

Silvennoinen JA, Karttunen TJ, Niemelä SE, Manelius JJ, Lehtola JK. A controlled study of bone mineral density in patients with inflammatory bowel disease. *Gut* 1995;37:71-76.

Simchuk EJ, Thirlby RC. Risk factors and true incidence of pouchitis in patients after ileal pouch-anal anastomosis. *World J Surg* 2000;24:851-856.

Sivakumar PV, Westrich GM, Kanaly S, Garka K, Born TL, Derry JM, Viney JL. Interleukin 18 is a primary mediator of the inflammation associated with dextran sulphate sodium induced colitis: blocking interleukin 18 attenuates intestinal damage. *Gut*. 2002;50:812-820.

Southey A, Tanaka S, Murakami T, Miyoshi H, Ishizuka T, Sugiura M, Kawashima K, Sugita T. Pathophysiological role of nitric oxide in rat experimental colitis. *Int J Immunopharmacol* 1997;19:669-676.

Srivastava ED, Barton JR, O'Mahony S, Phillips DI, Williams GT, Matthews N, Ferguson A, Rhodes J. Smoking, humoral immunity and ulcerative colitis. *Gut* 1991;32:1016-1019.

Stack WA, Long RG, Hawkey CJ. Short- and long-term outcome of patients treated with cyclosporin for severe acute ulcerative colitis. *Aliment Pharmacol Ther* 1998;12:973-978.

Stallmach A, Schafer F, Hoffmann S, Weber S, Muller-Molaian I, Schneider T, Kohne G, Ecker KW, Feifel G, Zeitz M. Increased state of activation of CD4 positive T cells and elevated interferon  $\gamma$  production in pouchitis. *Gut* 1998;43:499-505.

Summanen P, Baron EJ, Citron DM, Strong DM, Wexler HM, Finegold SM. *Wadsworth anaerobic bacteriology manual* 5<sup>th</sup> ed. Belmont, CA, Star publishing, 1993.

Sutherland L, Roth D, Beck P, May G, Makiyama K. Oral 5-aminosalicylic acid for inducing remission in ulcerative colitis (Cochrane Review). In: *The Cochrane Library*, Issue 1. Oxford: Update Software 2000.

Swidsinski A, Ladhoff A, Pernthaler A, Swidsinski S, Loening-Baucke V, Ortner M, Weber J, Hoffmann U, Schreiber S, Dietel M, Lochs H. Mucosal flora in inflammatory bowel disease. *Gastroenterology* 2002;122:44-54.

Szilagyí A. Review article: lactose – a potential prebiotic. *Aliment Pharmacol Ther* 2002;16: 1591-1602.

Talbot RW, Heppell J, Dozois RR, Beart RW Jr. Vascular complications of inflammatory bowel disease. *Mayo Clin Proc* 1986;61:140-5

Thomas GA, Rhodes J, Green JT, Richardson C. Role of smoking in inflammatory bowel disease: implications for therapy. *Postgrad Med J* 2000;76:273-79.

Thomas GA, Rhodes J, Green JT. Inflammatory bowel disease and smoking- a review. *Am J Gastroenterol* 1998;93:144-149.

Thompson-Fawcett MW, Marcus V, Redston M, Cohen Z, McLeod RS. Risk of dysplasia in long-term ileal pouches and pouches with chronic pouchitis. *Gastroenterology* 2001;121:275-281.

Tiainen J, Matikainen M. Long-term clinical outcome and anemia after restorative proctocolectomy for ulcerative colitis. *Scand J Gastroenterol* 2000;35: 1170-1173.

Tomita R, Tanjoh K. Role of nitric oxide in the colon of patients with ulcerative colitis. *World J Surg* 1998;22:88-91.

Toyoda H, Wang SJ, Yang HY, Redford A, Magalong D, Tyan D, McElree CK, Pressman SR, Shanahan F, Targan SR. Distinct associations of HLA class II genes with inflammatory bowel disease. *Gastroenterology* 1993;104:741-748.

Tromm A, Rickels K, Huppe D, Wiebe V, May B. Risk factors for osteoporosis (OP) in inflammatory bowel disease (IBD). *Gastroenterology* 1993;105:A542.

Tynkkynen S, Satokari R, Saarela M, Mattila-Sandholm T, Saxelin M. Comparison of ribotyping, randomly amplified polymorphic DNA analyses, and pulse-field gel electrophoresis in typing of *Lactobacillus rhamnosus* and *L.casei*. *Appl Environ Microbiol* 1999, 65:3908-3914.

Tysk C, Lindberg E, Jarnerot G, Floderus-Myrhed B. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. *Gut* 1988;29:990-996.

Ulisse S, Gionchetti P, D'Alo S, Russo FP, Pesce I, Ricci G, Rizzello F, Helwig U, Cifone MG, Campieri M, De Simone C. Expression of cytokines, inducible nitric oxide synthase, and matrix metalloproteinases in pouchitis: effects of probiotic treatment. *Am J Gastroenterol* 2001;96:2691-9.

Valkamo E. Ileostomy in ulcerative colitis. A long-term study of the results of conventional (Brooke's) and continent (Kock's) ileostomy in 161 patients. *Ann Chir Gynaecol Suppl* 1981;195:1-81.

Walmsley RS, Anthony A, Sim R, Pounder RE, Wakefield AJ. Absence of *Escherichia coli*, *Listeria monocytogenes*, and *Klebsiella pneumoniae* antigens within inflammatory bowel disease tissues. *J Clin Pathol* 1998;51:657-661.

Van Milligen de Wit AW, van Deventer SJ, Tytgat GN. Immunogenetic aspects of primary sclerosing cholangitis: Implications for therapeutic strategies. *Am J Gastroenterol* 1995;90: 893-900.

Watanabe T, Kubota Y, Muto T. Substance P containing nerve fibres in ulcerative colitis. *Int J Colorectal Dis* 1998;13:61-67.

Vecchi M, Bianchi MB, Calabresi C, Meucci G, Tatarella M, de Franchis R. Long-term observation of the perinuclear anti-cytoplasmic antibody status in ulcerative colitis patients. *Scand J Gastroenterol* 1998;33:170-173.

Veress B, Reinholt FP, Lindquist K, Löfberg R, Liljeqvist L. Long-term histomorphological surveillance of the pelvic ileal pouch: dysplasia develops in a subgroup of patients. *Gastroenterology* 1995;109:1090-1097.

Wischmeyer P, Pemberton JH, Phillips SF. Chronic pouchitis after ileal pouch-anal anastomosis: responses to butyrate and glutamine suppositories in a pilot study. *Mayo Clin Proc* 1993;68:978-981.

Yang H, McElree C, Roth MP, Shanahan F, Targan SR, Rotter JI. Familial empirical risks for inflammatory bowel disease: differences between Jews and non-Jews. *Gut* 1993;34:517-524.

Yasuda N, Thomas P, Ellis H, Herbst F, Nicholls J, Ciclitira. Perinuclear antineutrophil cytoplasmic antibodies in ulcerative colitis after restorative proctocolectomy do not correlate with the presence of pouchitis. *Scand J Gastroenterol* 1998;33: 509-513.

Zins BJ, Sandborn WJ, Penna CR, Landers CJ, Targan SR, Tremaine WJ, Wiesner RH, Dozois RR. Pouchitis disease course after orthotopic liver transplantation in patients with sclerosing cholangitis and ileal pouch anal anastomosis. *Am J Gastroenterol* 1995;90:2127-2181.

Zuccaro G Jr, Fazio VW, Church JM, Lavery IC, Ruderman WB, Farmer RG. Pouch ileitis. *Dig Dis Sci*. 1989;34:1505-1510.