Ulcerative Colitis – Surgery Outcome and Pathophysiological Aspects

Mattias Block

Department of Surgery
Institute of Clinical Sciences
Sahlgrenska Academy at University of Gothenburg

UNIVERSITY OF GOTHENBURG

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Front cover:

"3d rendered illustration of the colon"
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"I have kum to the konklusion that a good set of
bowels are more important to man than the brain"
-Anonymous

To my family and my friends

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Department of Surgery, Institute of Clinical Sciences
Sahlgrenska Academy at University of Gothenburg, Göteborg, Sweden

ABSTRACT

Background:
Ulcerative Colitis (UC) is a chronic inflammatory bowel disease; the etiology is mainly unknown. Around 30% of the patients are treated by surgery. The aims of this thesis were to evaluate outcome after specific surgical procedures and to investigate possible pathophysiological aspects.

Methods:
Functional outcome after ileal pouch-anal anastomosis (IPAA) and ileo-rectal anastomosis (IRA) was recorded by Öresland score; different pouch designs (*K* or *J*) were compared. Frequency of neoplasia in IPAA:s was evaluated in patients with previous neoplasias. Patients with UC and primary sclerosing cholangitis (PSC) were compared to patients with UC-only regarding outcome of IPAA or IRA. Galectin expression was investigated in full wall specimens from patients with UC.

Results:
IPAA:s with *K*-design and stapled anastomosis were associated with better Öresland score than IPAA:s with *J*-design. The obtained frequency of dysplasia in IPAA:s was 1.8% (95%-CI: 0-5.3%) in patients with previous neoplasia. Patients with UC-PSC operated on with IPAA have similar outcome as patients with UC-only, except for higher incidence of pouchitis. Patients with IRA in the same setting, have worse functional outcome and an increased rate of failure. There was no correlation between galectin expression and inflammatory grade.

Conclusions:
K-design was associated with best functional outcome; however, the study was non-randomized. The frequency of dysplasia after IPAA is low, even in a selected risk group. IPAA seems superior to IRA in patients with UC-PSC. The role of galectins in the pathogenesis of UC remains to be elucidated.

Keywords: Ulcerative Colitis, Long-term function, IPAA, IRA, Neoplasia, Surveillance, Primary Sclerosing Cholangitis, Galectins, Immunohistochemistry

LIST OF PAPERS

This thesis is based on the following publications and manuscript, which are referred to in the text by their Roman numerals (I-IV):

    I.   **Block M,** Börjesson L, Lindholm E, Öresland T
        Pouch Design and Long-term Functional Outcome after Ileal Pouch-Anal Anastomosis.
        Br J Surg. 2009 May; 96 (5): 527-32.

    II.   **Block M,** Börjesson L, Willén R, Bengtsson J, Lindholm E, Brevinge H, Saksena P
        Dysplasia or Cancer in the Colorectal Specimen in Patients with Ulcerative Colitis and Ileal Pouch-Anal Anastomosis – Rationale for Routine Surveillance?
        Submitted for publication in Journal of Crohn´s and Colitis.

    III.   **Block M**, Jørgensen KK, Lindholm E, Øresland T, Grzyb K, Smaastuen M, Vatn MH, Boberg KM, Börjesson L
        Colectomy for Patients with Ulcerative Colitis and Primary Sclerosing Cholangitis – what next?
        Journal of Crohn´s and Colitis 2013 Nov 14. doi: 10.1016/j.crohns.2013.10.008. [Epub ahead of print]

    IV.   **Block M,** Mölne J, Leffler H, Börjesson L, Breimer ME
        Immunohistochemical Studies on Galectin Expression in Colectomised Patients with Ulcerative Colitis.
        Submitted for publication in Histology and Histopathology.

ABBREVIATIONS

ATZ   Anal Transitional Zone

HGD   High-grade Dysplasia

HQoL   Health-related Quality of Life

IBD   Inflammatory Bowel Disease

IFD   Indefinite for Dysplasia

IPAA   Ileal Pouch-Anal Anastomosis

IRA   Ileorectal Anastomosis

LGD   Low-grade Dysplasia

PSC   Primary Sclerosing Cholangitis

QoL   Quality of Life

UC   Ulcerative Colitis

CONTENTS

v

INTRODUCTION

Background

"Morbid appearances in the intestine of Miss Bankes" is considered the first description of Ulcerative Colitis (UC) by Samuel Wilks (Fig. I), Guy Hospital, London, UK in 1859[1]. He did an autopsy of a 42 year-old woman who died after several months of diarrhoea and fever, demonstrating transmural ulcerative inflammation of the colon, differentiating it from bacterial dysentery.

UC is a chronic, relapsing inflammatory condition of the large intestine that affects individuals, often young, throughout life, although individuals of any age can be affected[2,3]. UC has an incidence in the Western world of 10-15/100.000 inhabitants and around 2.1 millions individuals are estimated to live with UC in Europe[4]. The onset is often between 20 and 35 years of age. The disease is slightly more common among females (1.2:1)[5]. The inflammation often begins in the rectum and spreads proximally in a continuous fashion[2]. Depending on the anatomic extent, patients can be classified as suffering from proctitis, left-sided colitis or pancolitits, the latter affecting almost the entire colon (Fig. II)[3]. Symptoms of UC are typically bloody diarrhoea, abdominal cramping and passage of pus or mucus; symptoms are often less severe in left-sided colitis and proctitis[3].

The aetiology and pathogenesis of UC are essentially unknown, but involves complex interactions between intestinal micro flora, host genetic- and immune factors, as well as environmental stimuli[6-13].

Figure I. Dr. Samuel Wilks. Courtesy US National Library of Medicine.

Up to 25-40% of patients with UC will develop extraintestinal manifestations that can affect various other organs such as joints, skin, bile ducts, eyes, lungs, and pancreas[14]. Most common are peripheral arthritis, erythema nodosum and pyoderma gangrenosum[14]. Anemia is also common, usually due to the combination of severe chronic inflammation and/or bleeding of the bowel. Extraintestinal manifestations usually respond to treatment of the inflamed bowel. There is, however, a need for multidisciplinary disposing of UC patients
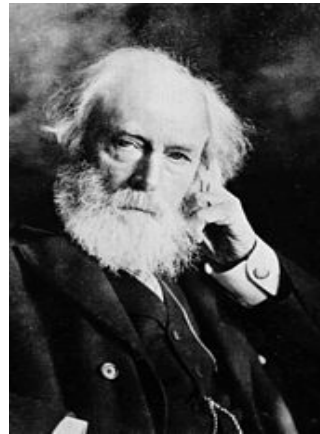
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Figure II. Different anatomical extent of UC; proctitis, proctosigmoiditis, and left-sided colitis. Pancolitis is inflammation beyond the middle of the transverse colon or of the entire colon. Courtesy US National Library of Medicine.

with extraintestinal manifestations involving surgeons, gastroenterologists, rheumatologists and others[14].

The total cost for healthcare (and sick leave) for UC is around 2.7-3.2 billions Euro/year in Europe[4]. The unemployment among the patients is around 10% and around 25% work part-time. Around 20% of the patients are early retired from their working life[4].

Pathophysiological Aspects

The etiology of UC involves an interaction between genetic- and non-genetic factors. The main theory of the pathophysiology of UC is an abnormal inflammatory response to bacterial contents in the intestinal lumen, in genetically predisponed patients[6-13].

Studies have identified 99 overlapping genetic risk loci for UC (28 shared with Crohn´s disease). However, the concordance rate in monozygotic twins is around 10-15%[15,16]. This observation favours the idea that non-genetic factors may play an important role in UC[17].

The intestinal mucosal cells act as a barrier, but they also care for delicate signal-functions between the host and the complex luminal milieu, dominated by bacteria. Maintaining a perfect balance between the two roles is central. Tight junctions between the mucosal cells[18,19], as well as the mucus layer, can be disrupted in UC[20,21]. These alterations can lead to translocation of bacteria from the lumen into underlying tissues and further to the circulation, creating a powerful inflammatory response[2,13]. The response leads to continuous epithelial damage, with erosions and ulcerations and a further breakdown of local defense mechanisms[22,23]. See Figure III.
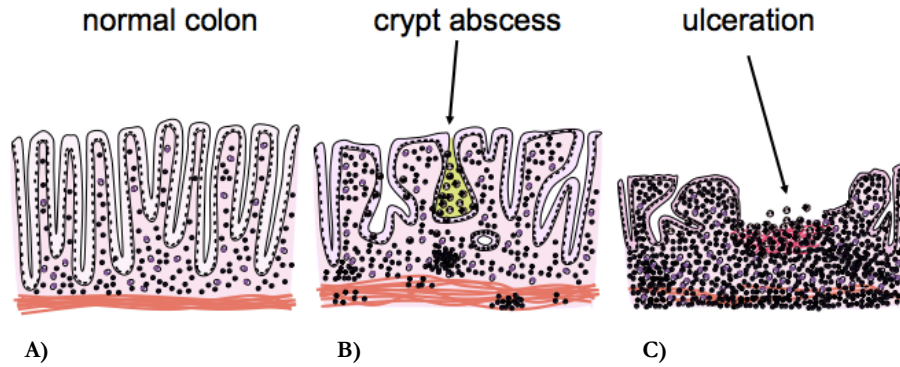
Figure III A-C. Colonic mucosa, from left to right; A) normal mucosa, B) UC; mild (cryptitis) and moderate (crypt abscess) inflammation, and C) severe (ulceration) inflammation

Typical for UC is infiltration into the lamina propria of innate immune cells (dendritic cells, macrophages and neutrophils), as well as adaptive immune cells (B- and T-cells)[24]. The increased number of active inflammatory cells result in an increased level of cytokines, interleukines and interferons[25].

Treatment

The first line of treatment is *pharmacological therapy*[26,27]. Corticosteroids and 5-ASA were the first pharmacological agents used[26,28]. Nowadays, more effective alternatives such as immunomodulators and biological agents (anti-TNF) are available. Although the pharmacological (non-surgical) treatment has improved over the years, around 30% of patients with UC will be operated upon during their lifetime[3,29]. There are two principal reasons for surgical treatment: 1) medically refractory disease in a chronic (50% of surgery) or acute (40%) setting, and 2) development of dysplasia or carcinoma (10%)[30]. Patients with extensive colitits are at higher risk of surgery (30%), compared to patients with left-sided colitis (5%), or proctitis alone (2%)[31]. Emergency surgery is indicated when the patient presents with a life-threatening complication, such as perforation, refractory bleeding, or toxic megacolon[32,33]. Since most patients have a long life expectancy, perfect surgical techniques are of outmost importance for assuring best possible outcome[34].

Surgery

When non-surgical treatment has failed and surgery is considered indicated, it is vital to address the patient with information of the surgical options and their expected outcome. Several aspects should be covered: postoperative complications, functional outcome, health-related quality of life (HQoL), reproduction, and the risk for proctitis/pouchitis[34,35]. Furthermore, the patients need to be informed about future medication, follow-up regims (considering the risk for carcinoma) and failure rates of surgical reconstructions[35-39].

Patients with UC, preferably young and active, usually have a strong desire for defecation through the normal route. Thus, the principal aim of the techniques that have evolved throughout the years, has been to offer most patients an option that not includes a conventional stoma.

The initial procedure in most patients is abdominal colectomy (removal of the entire colon to the rectum) and deviation with an ileostomy[30,32]. For the majority of patients, the final goal is to restitute bowel continuity and the main surgical options are ileo-rectal anastomosis (IRA)[40,41] or protectomy with ileal pouch-anal anastomosis (IPAA)[42,43]. The procedures can be done in one-, two- or three-steps.

Proctocolectomy with ileostomy[30], or proctocolectomy with continent ileostomy (CI; Kock pouch)[44] are other options. See Figure IV. Segemental colon resection has been reported[45] and can be associated with better functional outcome. However, the technique is not widespread[45].

Of outmost importance is the communication between the surgeon and the well-informed patient[32]. To date, there
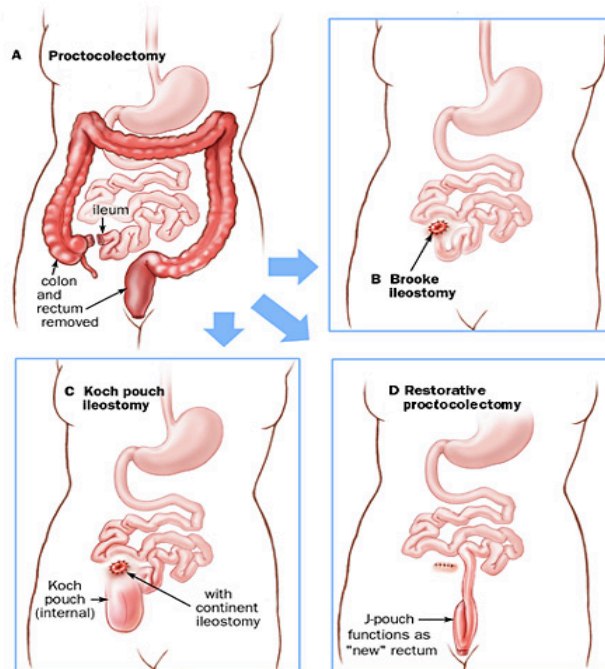

Figure IV. Surgical options for treatment of ulcerative colitis; A, proctocolectomy; B, Brooke ileostomy; C, Koch pouch (continent ileostomy); D, Ileal Pouch-anal Anastomosis (IPAA).

are no prospective, randomized trials comparing the main surgical options in UC.

Ileo-Rectal Anastomosis (IRA)

Lilienthal was the first surgeon who performed an IRA (or more common an ileo-sigmoidal anastomosis) for patients with UC, in the beginning of the 1900s[46]. See Figure V. This was considered a great innovation compared to previous surgical options, wich included appendicostomy (with irrigation), caecostomy or loop ileostomy. However, due to fear of the long-term risk of neoplasia and for the risk of poor functional outcome, IRA was never widespread. In the 50´s, Aylett[47] started to use the technique more extensively and he became a strong protagonist for IRA. Aylett reported an operative mortality of 5% and 90% of the patients were restored to health[48]. Others (among them Goligher), were very sceptic to the procedure[49]. In the late 70´s, Baker et al reported a carcinoma-risk of 6% after 20 years, increasing to 18% after 35 years[50]. In the early 90´s, Leijonmark reported no carcinoma after a mean follow up of 13 years[51]. However, failure rate was more than 50%, the majority due to recurrent inflammation of the rectum[51]. The risk for ongoing inflammation in the rectum/distal colon, for symptomatic relapse and for development of dysplasia and carcinoma, limited the use of IRA.

Figure V. Ileorectal anastomosis (IRA). Courtesy US National Library of Medicine.

The advantage of IRA compared to IPAA (see below) is less surgical trauma without pelvic dissection and thus a lesser risk for complications[52] such as incontinence, impotence, reduced fecundability and dyspareunia[41]. The challenge is the patient selection[36,46]. Most likely, compliance of the rectum is the most important factor[41,51]. When the rectum shows signs of severe inflammation and is non-compliant, or on the other hand shows a normal mucosa with good compliance, the choice of avoiding or performing an IRA is usually easy[36,46]. However, when the patient presents with moderate inflammation and some grade of decreased compliance, the management is more difficult[41]. Complications rates after IRA varies between 12 and 25%[40,41,52]. Failure rate nowdays is about 15-19% after five years and up to 30% after ten years[52-54]. If the IRA fails, one option can be IPAA.

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No study has compared IRA and IPAA in patients with UC with fecundability as primary endpoint. However, in a study of female patients with Familial Adenomatous Polyposis, the fecundity dropped to 54% after IPAA, while patients with IRA had the same fecundity as the background population[55]. In present-day Scandinavia, IRA is perhaps considered the main option for reconstruction of intestinal continuity[32,40,51,52,54], especially in women with the prospect of future pregnancy. Although IPAA is mostly advocated throughout the rest of the international surgical society, IRA is slowly becoming an alternative option, e.g. in Spain, but also in some parts of the UK.

Ileal Pouch-Anal Anastomosis (IPAA)

Since the early 80s, IPAA has been the prefered procedure for the majority of surgeons and the operation has evolved from a complicated, high-morbidity procedure to a fully evaluated and safe one[56]. The possibility of eliminating all diseased bowel and avoid a permanent ileostomy has apparent advantages compared to other options. Ravitch and Sabiston have been given the credit of pioneering this operation with experiments in dogs in
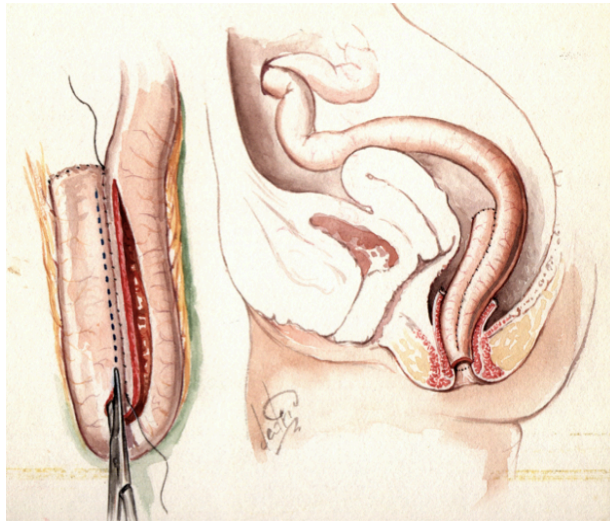
Figure VI. Construction of IPAA (in this case a J-pouch). The ileo-anal anastomosis is hand-sewn in this case.

1947[57]. Subsequent surgery in patients was however associated with severe morbidity and the authors felt obliged to advise against the procedure. Several surgeons tried to develop similar procedures during the 50s and 60s (Goligher, Drobni, Martin, Valiente). However, they still experienced major complications[58-60]. Only half of the patients achieved continence and around 40% eventually required a permanent ileostomy[59].

Parks and Nicholls presented the IPAA in 1978[42]. Functional outcome was reported as good, except for problems with pouch emptying; more than 50 % of the patients had to use a catheter for evacuation. This was due to the long pouch outlet in the initial design, the so-called S-pouch[61].
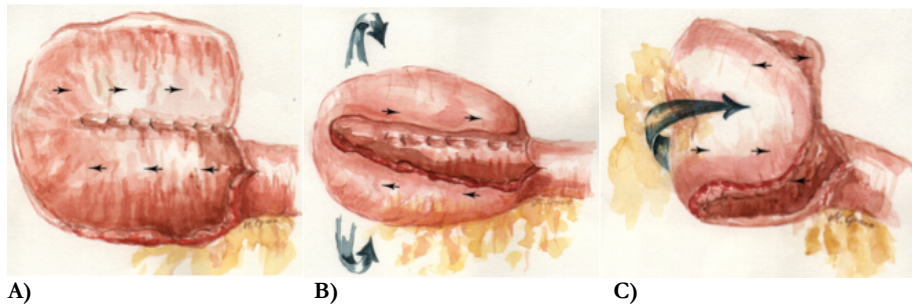
Figure VII. Construction of the IPAA (the pouch). A) First step. B) *J*-pouch. C) *K*-pouch.

Utsunomiya developed the *J*-pouch in the 1980s (Fig. VI and VIIB)[43]. The design was initially spread in Japan and in the US; the technique is the most commonly used today. The *W*-pouch (promoted by Nicholls) is associated with a larger pouch volume; however, the design includes additional intestinal length in the pouch[61].

Yet another design is to fold the pouch as a Kock pouch, omitting the nipple segment (see below; *K*-poch). The advantage of a *K*-pouch is that it develops a spherical design, resulting in a proportionally larger volume for the length of ileum used[62]. Furthermore, the *K*-pouch reduces the dead space in the small pelvis and there is some evidence that the *K*-pouch (as well as the *W*-pouch[56]) could have a slightly superior function. Usually, the IPAA is performed with a diverting temporary loop ileostomy as part of the procedure, but in selected cases, it may be omitted[63,64].

Hand-sewn versus Stapled Anastomosis

When performing an IPAA, two different techniques are used for the anastomosis between the pouch and the anus: hand-sewn anastomosis with mucosectomy, versus stapled anastomosis (Fig. VIII)[65,66]. Most studies show a slightly better functional outcome after stapled anastomosis[67,68], especially regarding continence[68,69]. The stapled technique is technically simpler, but patients may be at risk for developing cuffitis, ie symptomatic inflammation of the anal transitional zone (ATZ)[84, 86]. (The ATZ occupies the area from 1 to 1.5 cm above the lower border of the internal sphincter, and have squamous epithelium below and columnar epithelium above. Mainly, the ATZ is composed of transitional epithelium[70]. *This area will be referred to as the ATZ throughout this thesis.*)

Cuffitis, or strip proctitis, can be symptomatic in 25% of patients[71]. Theoretically, mucosectomy protects the patient from development of dysplasia, since all rectal mucosa is removed. However, several studys have shown that

this is not the case; mucosectomy does not completely protect patients from developing dysplasia in the ATZ[29,72,73] (See below "Histopathology and Neoplasia in IPAA").

A)   B)
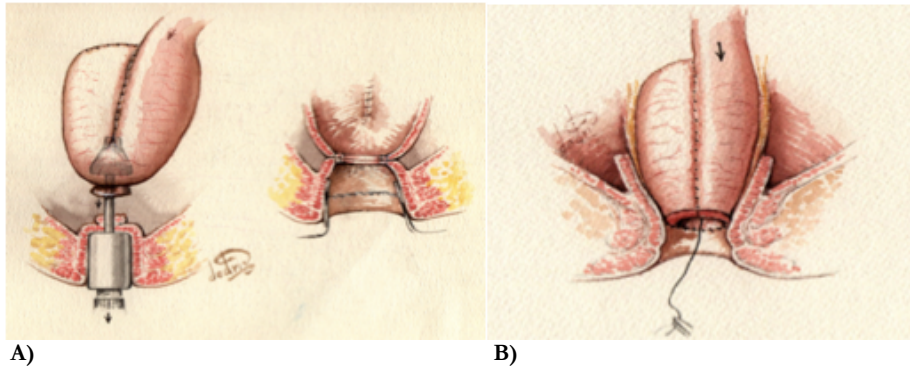
Figure VIII. Construction of IPAA (the ileo-anal anastomosis). A) Stapled anastomosis with a circular stapler-device. B) Hand-sewn anastomosis with mucosectomy.

Proctocolectomy and Ileostomy

Although IRA or IPAA are the main surgical options after abdominal colectomy, an alternative is proctocolectomy and conventional ileostomy. Historically, ileostomy with eversion of the mucosa was first described by Brooke in 1952[74], considerably improving quality of life for ostomists. Before that, ileostomies were associated with pronounced problems, mainly due to skin stenosis and poorly functioning stomal appliances. Compared to IPAA, the procedure is associated with lower risk for short- and long-term complications. Informed patients who choose a permanent ileostomy have a HQoL similar to patients with IPAA[75]. The procedure remains a valid option, preferably in patients with co-morbidity, not fit for IPAA. Furthermore, for some patients who prefer a stoma, or do not want to take the risks associated with IPAA (or IRA)[76].

Continent Ileostomy (Kock pouch)

The continent ileostomy (CI; Kock pouch) was developed by the Swedish surgeon Nils Kock and was first presented 1969[44]. See Figure IX. The CI is a well functioning alternative to a conventionel ileostomy. The major problem is the long-term need for surgical revisions (around 25% major revisions and 15% local procedures at 15 years[77]). The CI could be an option when there are technical difficulties in performing an IPAA (i.e. lack of bowel length) or

Figure IX. The continent ileostomy (CI; Kock pouch). By permission of stomavice.co.uk.

perianal disease, including fistulas and incontinence. An IPAA can sometimes be converted to a continent ileostomy; in a study from Sahlgrenska University Hospital on that procedure, 10/13 patients reported satisfactory function after median 6 years[78].

Complications after IPAA

An important aspect of IPAA is that the procedure is associated with some well-known complications; these can be separated into early or late. Early complications are common, with a range of 30-50% in large series[37,38,56]. In general terms, early complications do not differ from those seen after other major abdominal surgery. However, late complications (pouchitis, fistula, stricture of the ileo-anal anastomosis) are specific problems observed after IPAA. Both early and late complications can ultimately lead to pouch failure.

Early Complications:

Early complications after surgery are bleeding, anastomotic leak (4-7%)[56,79,80], pelvic abscess[35], intestinal obstruction (10-30%)[35,81,82] and stenosis of the ileo-anal anastomosis (8-22%)[35]. The complications should be treated according to general surgical principels. It is important to rule out a potential fistula before ileostomy closure. An abscess adjacent to the IPAA could affect long-term function and thus, rapid drainage is mandatory[37].

Late Complications:

Late complications can be defined as complications evident after closure of the diverting loop ileostomy, or after more than 90 days after IPAA[35]. These include

septic complications (pelvic sepsis or fistulas), stricture of the ileo-anal anastomosis, intestinal obstruction, pouchitis and pouch failure[34]. Neoplasia cannot be considered a complication to surgery, but rather a complication to the disease itself, (see below, Histopathology and Neoplasia in IPAA).

The incidence of pelvic sepsis varies from 5-40%[35,38,83-87], depending on definition and length of follow-up. Patient selection could affect the rate of pelvic sepsis; risk factors are ongoing treatment with steroids[83], Crohn´s disease[88] and perianal disease[88]. Several studies have shown that a septic complication (early or late) is a common reason for pouch failure[35,84,89]. Since septic complications can emerge surprisingly late after IPAA (years),[83,84] the patient condition, symptoms and type of complication affect timing and method of choice for treatment[84].

A feared late complication is fistulas, pouch-vaginal being the most common[90,91]. The majority of fistulas develop 6-24 months after surgery[88] and are mainly considered as a late complication. Around 50% of patients with pouch-vaginal fistulas eventually achieve recovery; however 25% still have fistulous discharge, but remain with the IPAA, whereas 25% need diversion or excision of the IPAA (failure)[92]. Other fistulas are pouch-perineal, pouch-cutaneous and pouch-presacral. Optional treatment varies from local repair to major surgical procedures (re-do). The reported succes rates after major surgery varies, but is around 50%, depending on type of fistula and extent of procedure[90,91,93].

Another late complication is development of an anastomotic sinus – a chronical cavity in the pelvis, almost mandatory behind the ileal pouch. Sinuses occur in 2-8% of patients[94]. Late stricture of the ileo-anal anastomosis is reported in 10-17%[35,80]. However, a report from the Mayo Clinic showed anastomotic strictures in around 40% after 20 years[36]. The majority of strictures are anastomotic webs that can be treated by digital or instrumental (Hegar) dilatation, often in office settings. A few patients require more extensive procedures[35]. Except for pouchitis, small bowel obstruction is the most common complication after IPAA; the reported incidence varies between 15 and 40%[35,36,81]. Around 25% of the patients need a surgical intervention, but the majority can be managed conservatively[81].

Most surgeons advocate a temporary loop ileostomy when performing an IPAA[95]. However, the loop ileostomy itself can be associated with problems. There is a readmission rate of 15%, mainly due to dehydration[96]. Furthermore, the closure is associated with complications. Wong et al[97] reported a morbidity

rate of around 10%, more than half of the morbidity was due to small bowel obstruction[97].

Pouchitis

Pouchitis, inflammation of the ileal reservoir, is the most common long-term complication, with a range of 30-50% depending on definition and follow-up time[38,98,99]. Hahnloser et al[36] showed that 40% of patients experienced at least one episode during the first 10 years after IPAA and the rate increased to 70% after 30 years[36]. The clinical features of pouchitis are similar to those of colitis and include frequent emptying of the pouch; the intestinal content is often more liquid and sometimes bloody. Faecal urgency, abdominal cramps and fever can also occur. At endoscopy, the typical features are erythema, erosions, friability, granularity, oedema and sometimes erosions (Fig. X)[100,101]. Histological examination of the pouch mucosa typically shows infiltrates of acute inflammatory cells, combined with signs of chronic inflammation[100]. Most patients experience short-lived pouchitis that respond promptly to treatment (see below). However, 10-20% develop recurrent, or chronic pouchitis[38,99].
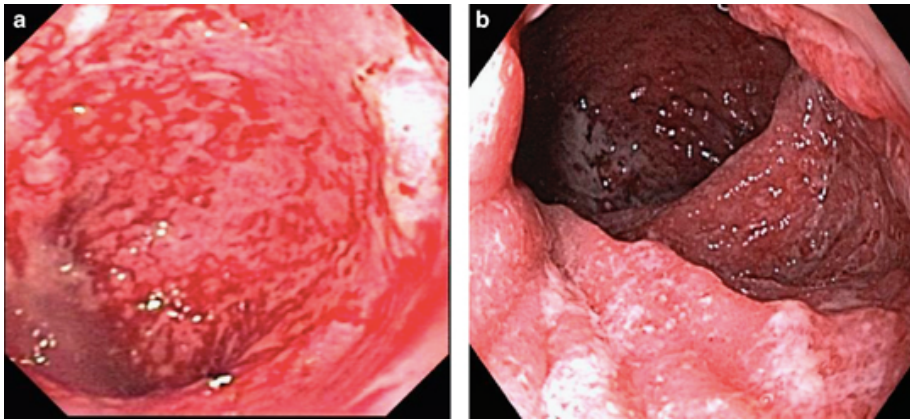
Figure X. Endoscopic pictures of ileal pouches with pouchitis. By permission of stomavice.co.uk.

Etiology

The etiology of pouchitis is still unknown. However, many theories have evolved throughout the years[102]. Initially, it was believed that fecal stasis with bacterial overgrowth was the basic mechanism[103], but this theory was later abandoned. Some authors have reported an increased bacterial count in patients with pouchitis[104], while others have not[105]. Cell turnover and metaplasia of the pouch has been observed, turning the ileum to a more colon-like tissue and

pouchitis could therefore resemble a "recurrence of UC"[106]. An argument against this theory is that pouchitis ususally responds to treatment with antibiotics[107]. Another theory is that bile salts, deconjugated by bacteria in the ileum, could increase the quantity of secondary bile acids in the pouch. These are cytotoxic, increasing the permeability of the pouch mucosa and this process could promote pouchitis[108,109]. Ischaemia of the pelvic pouch mucosa has been recorded[110] and proposed as a contributing factor to pouchitis. Pathophysiological mechanisms include potential production of free radicals, with secondary inflammation[111].

Some risk factors for development of chronic pouchitis have been proposed. These include back-wash ileitis[112], extensive colitis[113], primary sclerosing cholangitis (PSC, see below)[112], and use of non-steroidal anti-inflammatory drugs (NSAIDs)[114].

Diagnosis

The diagnosis of pouchitis should ideally be based on clinical, endoscopical and histological findings. The most well known tool for diagnosis is the Pouchitis Disease Activity Index (PDAI)[100]. This score includes three subscores: clinical symptoms, endoscopic findings, and histological changes[100]. Prompt response to treatment with antibiotics (below), together with clinical symptoms is regularly taken as diagnostic of pouchitis in clinical practice[101]. However, the risk with this empirical approach is that the incidence is overestimated[101].

Treatment

First-line therapy in patients with acute pouchitis is antibiotics. The majority of patients will have symptomatic relief in 1 to 2 days[107], and one to two weeks treatment is usually enough to achieve remission. Historically, Metronidazol has been the first choice[107,115,116] and it appears to be effective in 80-90%[117]. More recently, Ciprofloxacin has become more commonly used[118]. Metronidazol and Ciprofloxacin can be used in combination when monotherapy is insufficient[119]. However, some patients (10-20%) may require maintenance therapy with ongoing antibiotic treatment, either in low dose or at a reduced frequency[120].

Budenoside, cortocosteroids, sulphasalazine and 5-ASA have all been used in acute pouchitis, but not at long-term[120-122]. Infliximab has been used in chronic pouchitis[123-125]. However, patients included in the studys are few and long-term results are still lacking. Probiotics (VSL#3, a mixture of eight strains of probiotics) have been shown to be effective as matintenance therapy (antbiotics used to induce remission) and primary prophylaxis[117,126].

Still, the therapy of pouchitis remains mainly empiric. Randomized, multicentre, placebo-controlled, double-blinded, dose-ranging studies are warranted[127].

Pouch Failure

Failure of the pouch is defined as excision or diversion of the pouch, the latter varying in different studies from 1/2 to 2 years of time[35,36,128-130]. Failure rates varies among studies, but are usually around 5-7% at 5 years and 8-10% at 10 years[39,128,131,132] in patients with confirmed UC. In unselected cohorts, also including patients with CD and indeterminate colitis, the failure rate increases to around 35% after 10 years[133,134]. In cases where a secondary loop ileostomy is performed, the failure rate increases considerably (to around 65%)[135]. If the pouch has been defunctioned for more than one year, it is rarely reversed[136].

The most common reason for pouch failure is a septic complication (fistula, see also Complications above)[35,129,130,137]. Another common reason for failure is poor function (other reasons ruled out)[137,138]. Chronic pouchitis however, is a quite rare reason for failure (around 10%)[137].

In a retrospective study[139] including almost 2000 patients, four pre- and four postoperative factors were identified to be associated with pouch failure. Preoperative factors were: diagnosis, comorbidity, anal pathology and poor outcome of anal sphincter manometry. Postoperative factors were: anastomotic separation, anastomotic stricture, pelvic sepsis and fistula. The predictive risk for pouch failure at 1, 5 and 10 years was 0.1, 0.4 and 0.8%, respectively, in patients without risk factors. In patients with 6 risk factors however, the predicitve risk was estimated to 30, 70 and 90 %[139].

Pouch Function

Pouch function is a complex composite variable and can be considered the summary of several factors (anal sphincter function, pouch volume, pouch compliance, pouch emptying, small bowel function, pelvic volume, prescence of irritated bowel syndrome, psychological factors)[140]. In the literature, functional scores often evaluate the pouch function[36,129,141-144]. The scores can be used in clinical practice and usually include items associated with QoL[36,128,141,145] (see also Methods). Pouch function has been evaluated in individual patients over time and it seems to be stable, or marginally deteriorated[146]. Pouch function also correlates to HQoL[128,144].

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Pouch Physiology

Manovolumetry is a commonly used method for evaluation of pouch physiology (i.e. anal pressures and pouch volume/compliance)[143]. The major contributor to anal continence is the anal sphincter complex. The internal sphincter is a smooth muscle that contributes considerably to the anal resting pressure. It provides for passive continence and reduced resting pressure can be associated with leakage and soiling[140]. The voluntary control depends on the external sphincter muscle, the puborectalis muscle and the striated pelvic floor muscles. IPAA surgery regularly leads to reduction in anal pressure; some recovery is seen over time, however, usually not to preoperative levels[147-149].

Pouch volume and pouch compliance have been observed to affect pouch function; large pouch volume and high compliance leads to a lower bowel movement frequency and hence, a better functional outcome[143,150,151]. Reported volumes of the IPAA:s differ between studies with a range from around 150[61] to around 300 ml[152]. It has been proposed that pouch volume is the most important determinant for pouch function[143]. However, only around 20% of the variability of the functional score can be explained by volume and pouch compliance[143]. Some data indicate that compliance and volume is greater in pouches with W-design, compared to those with S- and J-design[61]. Interestingly, a recent study in patients with pouch dysfuncion suggested that compliance was not a contributing factor to the clinical problem[153].

From Dysplasia to Carcinoma

Malignancy develops by a multistep process, during which the normal cell progresses subsequently to a premalignant state (low-grade and high-grade dysplasia), carcinoma in situ, and further to carcinoma[154]. This is a multistep process associated with, and depending upon, defects in genetic controlling of normal cell proliferation and death[155]. Genomic instability leads to the generation of single and multiple aneuploid populations of cells. By further genetic changes, these populations eventually acquires the capacity for invasion[156].

In some diseases in the gastrointestinal tract, the axis inflammation-dysplasia-carcinoma is more or less evident. These include: esophagitis-Barret´s esophagus-esophageal carcinoma[157], primary sclerosing cholangitis-dysplasia-cholangiocarcinoma[158], ulcerative colitis-dysplasia-colonic carcinoma (Fig. XI)[159,160].

The cumulative incidence of dysplasia in UC varies between studies; one study showed 6% after median 20 years[161], while another showed 23.5% after 10

years[162]. The cumulative probability of carcinoma has been estimated to be 2% at 10 years, 8% at 20 years and 18% at 30 years[163].

Indefinite for dysplasia (IFD) is defined as cytoarchitectural alterations, including a spectrum of inflammatory and non-inflammatory changes, that do not reach the pathologists threshold for an unequivocal diagnos of true dysplasia (LGD or HGD)[164]. The clinical importance and outcome of IFD remains to be definied[165] (see below, Results and Comments, dysplasia).
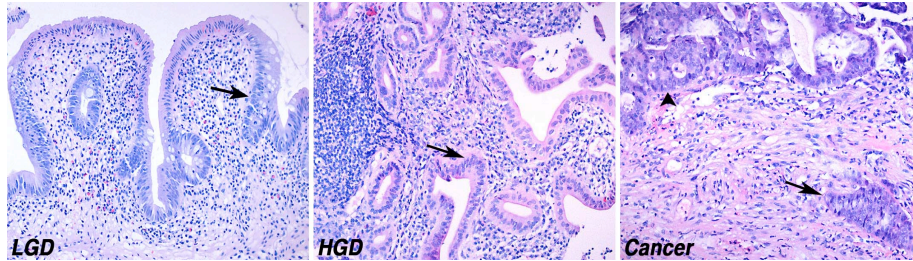
Figure XI. Light microscopy of biopsies from patients with ulcerative colitis and low-grade dysplasia (LGD), high-grade dysplasia (HGD) or invasive carcinoma. In LGD there is a loss of mucus content in epithelial cells and some nuclear atypia (arrow). In HGD there is a more prominent nuclear atypia (arrow) and a marked loss of tissue architecture. When invasive cancer is diagnosed, cells or glandular structures are invading the underlying tissue (arrow) and there is a marked cellular and structural atypia (arrowhead).

Histopathology and Neoplasia in IPAA

The assumed mechanisms for neoplasias (dysplasia or carcinoma) occurring in IPAA:s are based on the assumption that the axis chronic inflammation-dysplasia-carcinoma, can be applied[159]. In this context, it seems important to emphasize that the IPAA contains two different tissues: small bowel mucosa in the ileal pouch and rectal mucosa in the ATZ[70,166]. The inflammation-neoplasia axis can be adopted considering the ATZ[139,140,159,160]. However, the pathogenesis of neoplasias occuring in the ileal pouch is less clear. To date, 77 cases with dysplasia and 42 with carcinomas in IPAA:s, have been reported[29].

Proposed Risk Factors for Neoplasia in the IPAA.

Several factors have been purported to be associated with an increased risk for neoplasia in the IPAA. These include:

1) Previous dysplasia or carcinoma in resected specimen[29,70,72,73,167,168]

is suggested to be the most important risk factor[29,72,168,169] and in recent studies actually outpointed as the only one[72,73]. In the large study by Kariv et al[72], the

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adjusted hazard ratio for neoplasia was 3.62 (95% CI, 1.59-8.23) between patients with neoplasia in their specimen, versus patients without; 3.76 (95% CI, 1.39-10.19) was obtained in a similar study by Derikx et al[73]. Considering the ATZ, one study estimated the risk for neoplasia to be around 10% when dysplasia is present in the resected specimen and around 25% in cases with carcinoma, after a follow-up time of median 3 years[167].

2) Concurrent PSC[170-172]

Patients with IBD-PSC have an increased risk for neoplasia in the large bowel before colectomy[171]. Furthermore, it has been proposed that patients with UC-PSC have a higher risk for development of mucosal atrophy and neoplasia in the ileal pouch, compared to patients with UC-only[170]. Other studies have not been able to confirm these data[72,73].

3) Chronic pouchitis/type C mucosal changes in the ileal pouch[173]

In a large study from the Cleveland Clinic, chronic pouchitis was evaluated as a potential risk factor for neoplasia[72]. The adjusted hazard ratio obtained was 0.81 (0.28-2.31); thus, it seems unlikely that chronic pouchits is an independent risk factor.

4) Long duration of UC[172]

Longstanding colitis (10-20 years) has been proposed as an undependent risk factor[172]. Interestingly, in a study on 26 patients with carcinoma in IPAA:s, all patients had a disease history of 10 years, or more[29]. However, Kariv et al did not identify duration of UC as an independent risk factor (adjusted hazard ratio 1.01 [0.97-1.05])[72].

Dysplasia in the Ileal Pouch:

Until now, 77 cases of dysplasia in the IPAA have been reported[29]; 49 were LGD, 15 HGD and 13 unspecified dysplasia. The locations were 28 (36%) in the pouch body, 6 (8%) in an unspecified location in the pouch and 2 (3%) at both ATZ and in the pouch. (ATZ was the location in 41 (53%)[29] cases, see below.) Different rates of prevalence for dysplasia in the pouch have been reported by different authors, from low prevalences (0.6%-0.9%)[72,73,174,175] to higher ones (7.5%-19%)[170,176,177] (See Table 1).

Table 1. Cases with dysplasia in the ileal pouch (2000-).

Reference	No	Duration IBD, y	Duration follow-up, y	Dysplasia [n]	Prevalence
Thompson-Fawcett 2001[175]	106	NA	2-22	1 LGD	0.9%
Hultén 2002[176]	40 CI	NA	26-34 med 30	3/2 LGD*	7.5%/5%*
Herline 2003[174]	160	NA	4-13	1 LGD	0.6%
Stahlberg 2003[170]	16 PSC	9-13	4-20	3 LGD, 1 HGD	LGD 19% HGD 6%
Börjesson 2004[177]	45	1-28 med 6	17-46 med 25	2/0 LGD*	4.5%/0*
Elkowitz 2004[178]	30	0.5-21 med 3	1-5 med 3	2 aneuploidy	6.7%
Hernandez 2010[179]	38	1-11	3, 6 and 12 mo post-op	1 LGD	2.6%

y, years; NA, not available; med, median; mo, months; post-op, postoperative.
* Two independent pathologists.

Dysplasia in the ATZ

It should be emphasised that the proctocolectomy preceeding the IPAA substantially reduces the risk for UC-associated neoplasia. However, the procedure does not completely void the risk. When a stapled anastomosis is employed, there is a cuff of rectal mucosa (0.5-2 cm) left behind; this tissue of mucosa has the potential to develop inflammation and neoplasia[70]. In a hand-sewn anastomosis, preceeded by mucosectomy, remnants of rectal mucosa (islets) have been found in as much as 20% of the cases[180,181]. Among 77 reports of dysplasia in IPAA, the location was ATZ in 41 (53%)[29]. As for the pouch, the prevalences varies between studies, from 0%-0.7%[72,73,182,183] to 2.9%-3.4%[70,178,184] (See Table 2).

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Table 2. Cases with dysplasia in the ATZ (2000-).

Reference	No	Duration IBD, y	Duration follow-up, y	Dysplasia	Prevalence
Thompson-Fawcett 2000[185]	113	0.3-40 med 7	0-10 med 2.5	1 aneuploidy	0.9%
O´Riordian 2000[184]	210	0.2-38 med 7	5-10 med 6.5	6 LGD 1 HGD	LGD 2.9% HGD 0.5%
Remzi 2003[70]	178	NA	12-13 med 10.8	6 LGD 2 HGD	LGD 3.4% HGD 1.1%
Coull 2003[182]	110	2-32 med 9	1-12 med 4.5	0	0
Saigusa 2003[183]	91	0.3-42 med 10	0.2-9 med 3	0	0
Pishori 2004[186]	303	0.2-18 med 9	med 3.5	2 dysplasia	0.7%
Elkowitz 2004[178]	30	0.5-21 med 3	1-5 med 3	1 aneuploidy	3.3%

y, years; NA, not available; med, median

Carcinomas in IPAA

Until recently, 42 cases with carcinomas in IPAA:s have been reported[29]; 11 were reported from the same group[72]. Eight (19%) cases have been found in the pouch body, 4 (10%) in unspecified location of the pouch, 1 (2%) in the afferent limb and finally, 2 (5%) in the ATZ and pouch body together. However, the majority (27; 64%) was localised in the ATZ only[29]. Furthermore, in the recent nationwide study from Holland, the majority of carcinomas (10/16; 63%) was in the ATZ[73]. Interestingly, the literature reveals that the majority of patients with carcinomas actually had mucosectomy and hand-sewn anastomosis. The reasons for this could be length of follow-up (all anastomoses were hand-sewn in the beginning of IPAA surgery)[187] and that hand-sewn anastomoses preferentially were performed in patients with risk factors[169].

Surveillance for Neoplasia in IPAA

Although the cases of carcinomas in IPAA:s are few, the reports have started a discussion on the place for surveillance[72,73,188]. Recent guidelines from the European Crohn´s and Colitis Organisation (ECCO) suggest surveillance for patients with neoplasia in the (procto)colectomy specimen and for patients with PSC[188].

Primary Sclerosing Cholangitis (PSC)

First described by Smith and Loe[189], primary sclerosing cholangitis (PSC) is a chronic, progressive inflammatory disease in the biliary tree, associated with formation of strictures, fibrosis and destruction of intra and/or extrahepatic bile ducts[190]. PSC can deteriorate liver-function and lead to end-stage liver disease[190,191]. The specific ethiology of PSC is unknown, interactions between environmental factors and genetic variations are components of theories regularly proposed[192].

PSC affects males more than females (2:1). The incidence is 0.9-1.3/100 000 person-years and the prevalence is 8.5-13.5/100 000[193,194]. A recent study from the western health care region (Västra Götaland), Sweden, showed a prevalence of 16/100 000 person-year[195]. Interestingly, this is the highest prevalence reported to date.

The clinical course of PSC in a single patient is difficult to predict and can vary from asymptomatic disease to fulminant cirrhosis and liver failure[196]. The most feared complication is cholangiocarcinoma[196]. There is no medical treatment for PSC and the only curative treatment is liver transplantation[191,196].

PSC is associated with UC in up to 75 % of patients in Northern Europe[190,197] and around 5% of patients with UC will develop PSC[198]. In concordance with UC, PSC does not seem to be a classic autoimmune disease. The association between PSC and UC is not fully understood[199]. Interestingly, similar as for UC, smoking has a protective effect on PSC[192,200].

Surgery for UC in Patients with PSC

It is important to be aware of that (procto)colectomy does not affect the progression of the liver disease[201]. The course of colitis in patients with UC and PSC (UC-PSC) is different from that in patients with UC without PSC (UC-only). Patients with UC-PSC usually have a more quiescent clinical course, increased incidence of pancolitis and a higher risk for dysplasia and carcinoma[202,203]. As a consequence, the prerequisites for surgical treatment differ from those for patients with UC-only. Patients with UC-PSC have a higher rate of colectomy[204], dysplasia and carcinoma are more common indications[171].

Surgical options for patients with UC-PSC are basically the same as for patients with UC-only. However, for the vast majority of patients with UC-PSC, IPAA is going to be advocated[205,206]. Proctocolectomy and ileostomy can be associated with massive bleedings from peristomal veins/varices (in around 25% of patients)[205], a complication very diffcult to treat[205,207]. The use of IRA in UC has increased (in Scandinavia) over the last decades; however, the literature on

patients with UC-PSC and IRA is sparse. Although IPAA is the preferred choice, several studies have found that patients with UC-PSC are at a higher risk for development of pouchitis[205,208-210], while others did not[211,212].

Few studies have reported on functional results after IPAA in patients with UC-PSC; however, the outcome seems to be similar to patients with UC-only[206,211,213]. Interestingly, there is no study on QoL after colorectal surgery for patients with UC-PSC.

Galectins-Potential Role in the Pathophysiology of UC

As described above, the pathophysiology of UC is very complex and essentially unknown. Since UC is characterized by an inflammatory process, it is of interest to explore different factors that contribute to the regulation of the inflammatory process. One such factor is the mammalian galectin. Galectins are a family of small soluble carbohydrate-binding proteins (lectins) that participate in a large number of biological processes such as development and progression of carcinoma[214,215] as well as immunoregulatory effects[215,216]. For these reasons, galectins represent potential targets for therapeutic intervention of disease[217-219].

Galectins contain a conserved carbohydrate recognition domain (CRD) composed of 135 amino acid residues with affinity for β-galactosides as found in glycoproteins and glycolipids[220]. They are synthesized in the cytosol and have functions intracellularly (cytosol and nucleus) as well as in extracellular compartments (cell-cell and cell-matrix adhesion, regulating cell survival and signalling, cell differentation, influencing chemotaxis, or interfering with cytokine secretion affecting the immune regulation)[221,222]. Galectins interact with carbohydrates wich exist in different forms on almost all cell-surfaces. Also the extracellular matrix (ECM) contains large amounts of carbohydrates, mainly glycoproteins[223]. Carbohydrates are involved in many biological processes, and play potential important roles in several disorders and diseases[223,224]. The ability of galectin CRDs to cross-link the lactoseamine unit (carbohydrate + amino acid) within surface glycoreceptors allows galectins to participate in several immune responses[220].

The mammalian galectin family consists of 15 proteins and are subdived into three subgroups depending on the protein architecture; i) the prototype galectins (galectins-1, -2, -5, -7, -10, -11, -13, -14 and -15), containing one CRD wich can occur as monomers or as dimers, ii)
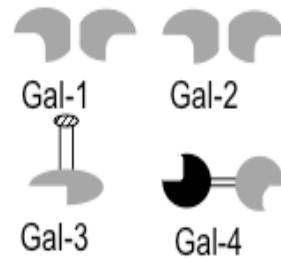
Figure XII. Galectins-1 to -4. From Leffler et al.

chimera-type galectins (galectin-3), wich contains one CRD and one non-lectin N-terminal domain, occuring as monomers, dimers, or higher order oligomers and iii) the tandem repeat-type galectins (galectin-4, -6, -8, -9 and -12), containing two distinct CRDs connected by a short linker region[220,225]. See Figure XII.

Galectins 1-4 in the Gastrointestinal Tract

Nine galectins (-1, -2, -3, -4, -6, -7, -8, -9, and -15) are expressed in the mammal digestive tract. Galectins-1 to -4 have all been observed within the human gastrointestinal tract, both in the normal digestive tract[226] as well as in the diseased intestine[226-228]. Galectins-1 and -2 are evolutionary related paralogues of a prototype galectin[229]. The most prominent galectins in the intestinal epithelial cells are galectins-3 and -4. Figures XIII-XIV shows the expression patterns of galectins 2-4 in normal and inflamed colonic tissue, below are the distribution and function of different galectins described more in detail.
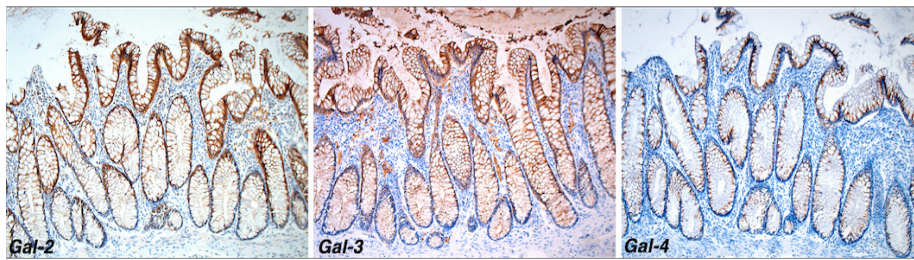
Figure XIII. Expression of galectin-2, -3 and -4 in normal colon tissue.
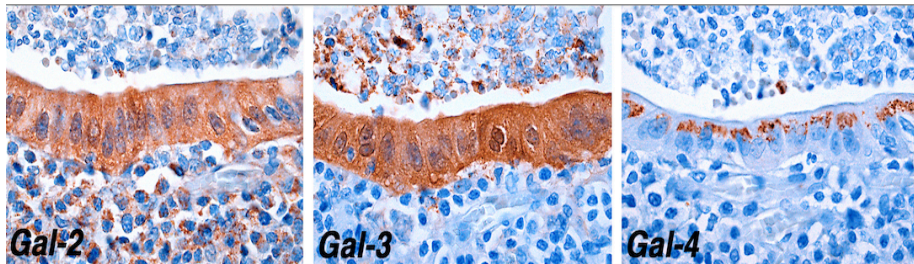
Figure XIV. Expression of galectin-2, -3 and -4 in inflamed colonic tissue. Galectins -2 and -3 stain the entire cytoplasm when the epithelial cells are devoid of mucose. Galectin-4 is only present in a supranuclear position.

Galectin-1 is mainly anti-inflammatory by induction of apoptosis in T-cells responsible for production of IFN-g[227,230]. Galectin-1 contributes to activation of B-cells[231] but can, in contrast, negatively regulates B-cell proliferation and it´s signal transduction[232]. Galectin-1 also affects the physiology of monocytes and

macrophages through effects on antigen presentation and phagocytosis[233]. In colonic carcinoma, increased expression of galectin-1 has been associated with neoplastic progression[224,234].

Galectin-2 has been studied less but found mainly to be anti-inflammatory and supporting wound healing in the intestine[235-237]. The mechanism is induction of apoptosis of activated T-cells[238] and inhibition of pro-inflammatory cytokines release (IL-6, IL-12p70)[237].

The most thouroughly studied galectin in humans is *galectin-3*, first defined as a surface marker on macrophages[239], then been found in many cell types, including eosiniphils, lymphocytes, mast cells, neutrophils and activated epithelial cells[240]. Galectin-3 is highly expressed in macrophages and is mainly pro-inflammatory, promoting neutrophil and monocyte adhesion to laminin and endothelial cells[241], but has also regulatory and tissue protecting effects[220]. Down-regulation of galectin-3 in intestinal epithelium in IBD has been reported[228], possibly involved in the induction of fibrosis. Galectin-3 is used in clinical histopathology, particularly in diagnosing thyroid tumors[242]. Increased level of galectin-3 has been observed in colonic carcinoma and correlates with neoplastic progression[224,243-245].

Galectin-4 is highly and specifically expressed in intestinal epithelial cells[246]. In IBD, galectin-4 has the most distinct pathogenic role as a specific activator of intestinal CD4+ T-cells[247], stimulating IL-6 production, an inflammatory cytokine contributing to progression of colitis. Increased expression of galectin-4 has been observed in colorectal carcinoma, suggesting a prognostic value for galectin-4[234].

Studies on Galectins in IBD

Various aspects for the roles of galectins in IBD pathogenesis (as well as in colorectal adenoma/carcinoma) have been studied, see Table 3. Most of these have been studied in various experimental animal models[227], both pro- and anti-inflammatory properties of various galectins have been identified[228,235,236,248]. Previous studies have implicated galectins in patients with IBD as potential markers of disease as well as potential theurapeutic agents[230,249]. However, the clinical significance in the human situation is uncertain. Previous studies on humans are limited regarding the number of patients included and their clinical status[250,251].

Table 3. Galectins studied in the human gastrointestinal tract.

Galectin	Disease	Alteration	Clinical implication	Refs
1	Colorectal adenoma and carcinoma	Increased expression	Prognostic value	224,234,243
	IBD	Increased expression and high IL-10	Possible treatment	230,249
2	IBD	Increased expression	Supress inflammation	235,237
3	Colorectal adenoma and carcinoma	Increased expression	Prognostic value, clinical stage	224,243-245
		Decreased expression	Metastatic capability	252
	IBD	Increased and decreased expression	Pro- and anti-inflammatory Fibrosis	228,240,253,254
4	Colorectal carcinoma	Increased expression	Prognostic value	234
	IBD	Increased expression	Enhance inflammation	247

AIM

Ulcerative Colitis is a complex disease, often with onset at young age. A deeper understanding of the pathophysiology, clinical course and treatment strategies is crucial for optimal care of patients. The overall objective of this thesis was to further extend the knowledge in the area of pathophysiology and the outcome of surgery.

The specific aims of the included studies were to investigate:

- Outcome of different IPAA-designs on long-term IPAA function (I).

- The frequency of neoplasia in patients with IPAA and foregoing neoplasia in their colorectal specimen (II).

- Outcome of IPAA and IRA in patients with UC and concurrent PSC (III).

- If the galectin expression in the colonic intestinal epithelium correlates to the severity of the inflammatory response (IV).

PATIENTS

Paper I
This study included 619 patients from the Swedish Surgery for Colitis Register, operated on at Sahlgrenska University Hospital 1982 – 2004. All patients had IPAA. Five hundred and fifty-six patients had either *J*- or *K*-pouch and were included in the study. Four hundred and twelve (87%) returned the questionnaire.

Paper II
Six hundred and twenty-nine patients from the Swedish Surgery for Colitis Register were operated on with IPAA in Gothenburg, 1982 – 2010. Patients with dysplasia or carcinoma in their colorectal specimen were identified. Ninety patients were included in the study, 56 completed the study protocol.

Paper III
The study group (UC-PSC) comprised patients with UC and PSC from the PSC database, Rikshospitalet, Oslo, Norway. All patients (n=48) had colectomy or proctocolectomy and subsequently IPAA (n=31) or IRA (n=17).
The control group (UC-only) was recruited from the Swedish Surgery for Colitis Register; all patients (with UC, but without PSC) were operated on at Sahlgrenska University Hospital, Gothenburg. One hundred and thirteen (62 IPAA and 51 IRA) patients were included. Patients with IPAA (in ratio 2:1 to the study group), with similar age at colectomy and the same gender as the patients with IPAA in the study group, were randomly chosen as controls. For patients with IRA, 57 consecutive patients, operated during the same time frame (1995–2007) as the patients in the study group, were included. Two patients had emigrated, leaving 55 available for analysis.

Paper IV
Twenty-two (14 males, 8 females) patients with UC, scheduled for bowel resection, were included prospectively. Ten patients (8 right-sided hemicolectomies for colonic carcinoma, 2 sigmoid resections for volvulus) were enrolled as controls.

METHODOLOGICAL CONSIDERATIONS

Swedish Surgery for Colitis Register (I, II, III)

Since 1982, consecutive data on patients operated on with IPAA at Sahlgrenska University Hospital, Gothenburg have been registered. The Register includes patient demographics, diagnoses (UC or CD), data on surgical procedures, histopathology, functional outcome, complications, and failure. Patients with IRA are included since 1995. The Register is now part of The Swedish IBD Registry – SWIBREG, wich today includes around 23 000 patients. The register has been validated regularly throughout the years.

The Norwegian PSC Register (III)

The Norwegian PSC Register is a database that contains data on around 450 patients with PSC admitted to the Section for Gastroenterology, Oslo University Hospital, Rikshospitalet, Oslo, Norway. The register includes all PSC-patients from the Oslo area, but also patients who were referred from all over Norway. Since the department is the only liver transplant centre in Norway, all Norwegian patients evaluated for liver transplantation, are included in the Register.

Pouch Functional Score (Öresland score; I, III)

Pouch function was assessed with a score that was developed and has been extensively used in our institution for more than 20 years[255]. The score includes 10 items, summarized to a score (0-15, 15 worst).

In an attempt to validate the score, 60 patients completed the score-questionnaire, and plotted their self-experienced function on a visual-analogue scale. The correlation between the two recordings was r=0.55, p<0.001. The patients had a tendency to grade better on the VAS-scale than on the Öresland score[256]. Another study from our institution showed that a score>8 is correlated to a reduced HQoL[128].

Table 4. Öresland score (Pouch Functional score)

Score items		Score points		
		0	1	2
No of bowel movements	Day	≤4	5	≥6
	Night	0	>1/week	≥2/night
Urgency		No	Yes	
Evacuation difficulties		No	Yes	
Soiling, seepage	Day	No	>1/week	
	Night	No	>1/week	
Perianal soreness		No	Occasional	Permanent
Protective pad	Day	No	>1/week	
	Night	No	>1/week	
Dietary restrictions		No	Yes	
Medications		No	Yes	
Social handicap		No	Yes	

Throughout the years, several scores for evaluation of IPAA function have been proposed. Lovegrove et al developed a score, PFS (Pouch Functional Score), with items only related to HQoL (Cleveland Global Quality of Life)[144]. Each item was given a weight that correlated to the impact on HQoL. Several of the included items are similar to the ones in the Öresland score (bowel frequency, grade of incontinence, urgency, and use of medication). However, some items are not included in the PFS (evacuation difficulties, perianal soreness, diet and social restrictions)[144].

In a recent study from Aarhus, Denmark[142], a score including 13 items, was proposed. The score is similar to the Öresland score, but also includes stool concistency, incomplete evacuation and need for antibiotic treatment. The score does not include social handicap, but has been correlated with QoL in a multivariate model[142]. Urgency, incomplete emptying, number of bowel movements/24 hours, major incontinence and use of anti-diarrhoeal mediciation was found to have a significant impact on QoL[142]. The score was subsequently used in a study on all patients (1047) in Denmark, operated on with IPAA for UC 1980-2010[257].

Rectal Function (III)

Rectal function was evaluated by the Öresland score. As written above, the score is tailored for patients with IPAA, but has been used before for evaluation of patients with IRA for UC[40].

Endoscopy & Biopsy (II)

Olympus video-gastroscopes were used for macroscopic evaluation and mucosal biopsies of the ileal pouches. The pouch mucosa was evaluated according to PDAI[100] and included assessment of edema, friability, granularity, loss of vascular pattern, edema, polyps, lesions and ulcerations. A rigid pediatric anoscope was used for evaluation and biopsies at the ATZ.

The majority of the examinations were conducted by the same investigator (MB). Four biopsies were collected from each pouch and two from the ATZ. Collecting biopsies from the ileal pouch is a relatively straight forward procedure. In the ATZ however, the manoeuvre is technically demanding and often associated with a considerable discomfort for the patient, especially in patiens with hand-sewn anastomoses[69].

Where and how biopsies from the IPAA should be collected depends on the aim of the procedure. ECCO guidelines for surveillance do not provide further details. Four to 6 biopsies from the ATZ and 2 to 6 from the pouch body and afferent limb have been suggested [29].

Histopathology (II, IV)

In paper II, the biopsies were fixed in formalin, embedded in paraffin after dehydration and cut at a thickness of 3-4 μ, stained with haematoxylin-eosin and in addition PAS to illustrate mucins. Biopsies from the ileal pouches were reviewed according to established criterias for mucosal adaptation, chronic/acute inflammation[258] and dysplasia[259]. Biopsies from the ATZ:s were reviewed for chronic inflammation[258] and dysplasia[259]. Dysplasia was graded as no dysplasia, indefinite for dysplasia (IFD), low-grade dysplasia (LGD) and high-grade dysplasia (HGD)[259]. There are well-known difficulties and controversies about the evaluation of dysplasia in the presence of inflammation[173,176,259-262]. A wide interobserver variation has been found between pathologists[260,261,263] and the consistency appears to be poor, especially in grading of IFD and LGD[150,176,73,165,260]. In a recent study by Derikx et al, a histopathologic reassessement of 29 cases was performed; 22 (76%) cases shifted grad (18 were downgraded and 4 upgraded)[73]. Therefore, two experienced pathologists with special interest in the gastrointestinal tract reviewed the biopsies independently.

In paper IV, the colorectal specimens were collected at regular intervals according to a standardized protocol from each colonic and rectal area. In addition, specimens were collected separately from macroscopic lesions and ulcerations areas, if present. The specimens were fixated in formalin and embedded in paraffin. Inflammation grade was evaluated after staining with haematoxylin-eosin. The inflammatory activity was graded as mild (cryptitis), moderate (crypt abscesses) or severe (ulcerations). Leukocyte subtypes were identified by morphology (neutrophilic granulocytes and lymphocytes) and, in addition, by cell-specific monoclonal antibodies; CD3 (T-cells), CD68 (macrophages) and CD138 (plasma cells).

Pouchitis Disease Activity Index (PDAI; III)

PDAI is the most well known score for grading of pouchitis. This 18-point score was developed by Sandborn et al in 1994[100] and consists of a triad domains: 1) clinical symptoms, 2) endoscopic findings and 3) histological changes. Each subscore ranges from 0-6 (6 worst), with a maximum summoned score of 18.

Another widespread classification of pouchitis is Moskowitz (St Marks) criteria[258]. The score consist of histopathological gradings only and scores acute inflammation (0-6) and chronic inflammation (0-6). Thus, PDAI only evaluates acute inflammation (PMN infiltrates and ulcerations), whereas Moskowitz[258] also evaluates chronic inflammation (inflammatory infiltrates [lymphocytes] and villous atrophy). Some authors recommend that studies refer to both indices[264].

Yet another score that evaluates chronic inflammatory changes is the Heidelberg score[265]. The Heidelberg score and the PDAI score were validated in 41 patients[265]. Considering both scores, there seem to be a low correlation between the symptom sub-score and endoscopy/histopathlogy. Therefore, it was proposed that pouchitis could be diagnosed only by endoscopy and histopathology (omitting the symptom component)[266]. Others however, have proposed that the histopathological component should be left out[101]. Several studies use a clinical definition (usually typical symtoms and/or prompt response to antibiotic treatment) of pouchitis[208,209,212].

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Proctitis (III)

In patients with UC-PSC and IRA, proctitits was recorded at scheduled follow-up visits that included endoscopy of the rectum with a flexible endoscope; one endoscopist evaluated all patients. The macroscopic appearance of the mucosa was graded according to the Mayo score[267]. Six biopsies were taken from each patient. Histopathological inflammation was graded according to established criteria[268] (absent, mild, moderate or severe)[268].

Surgical Complications after IPAA or IRA (III)

Surgical complications were defined as any complication after IPAA or IRA demanding an endoscopic or surgical intervention. Recorded complications were: bleeding, anastomotic dehiscence, anastomotic stricture, small bowel obstruction, pelvic sepsis, or fistula. In patients with UC-PSC, complications were registered prospectively during scheduled follow-up visits. In patients with UC-only, complicatons were recorded by data from the Swedish Surgery for Colitis Register and from review of medical records.

The analysis of patients with UC-only has weaknesses since a valid retrospective recording of surgical complications is difficult to achieve. However, the items (associated with an endoscopic or surgical intervention) selected for analysis in the study were robustly addressed in the patients' medical records. The most validated method to record surgical complications is Clavien-Dindo grading system[269]. The score is divided into 7 groups ranging from any deviation to intervention under anesthesia, life-threatening complication, failure and eventually death[269].

Failure of IPAA or IRA (III)

Failure of the IPAA was defined as excision of the pouch or diversion with a proximal stoma for more than one year[36,129,131,137]. Failure of the IRA was defined as proctectomy or diversion for more than one year[40,52,54]. The main reason (according to the Register/medical records) for failure was recorded.

Immunohistochemistry (IV)

We used an automated immunohistochemical staining method, the EnVision™ Flex high pH (Link) detection kit (Dako K8000, Copenhagen, Denmark). This is a common technique in modern pathology, using immunohistochemistry as a diagnostic tool. It is used both in clinical practise and in research. For principal

steps of the technique, see Fig. XV. All specimens were coded. Specimens were analysed in a blinded fashion by two investigators. Galectin-1, -2, -3 and -4 expressions were examined and staining intensity was recorded on a 4-level scale; negative (0), trace amounts (1), weakly positive (2) and strongly positive (3). Focal pattern was assigned as focal (f). The intensity was analysed in different areas of inflammatory activity as well as in control tissue.
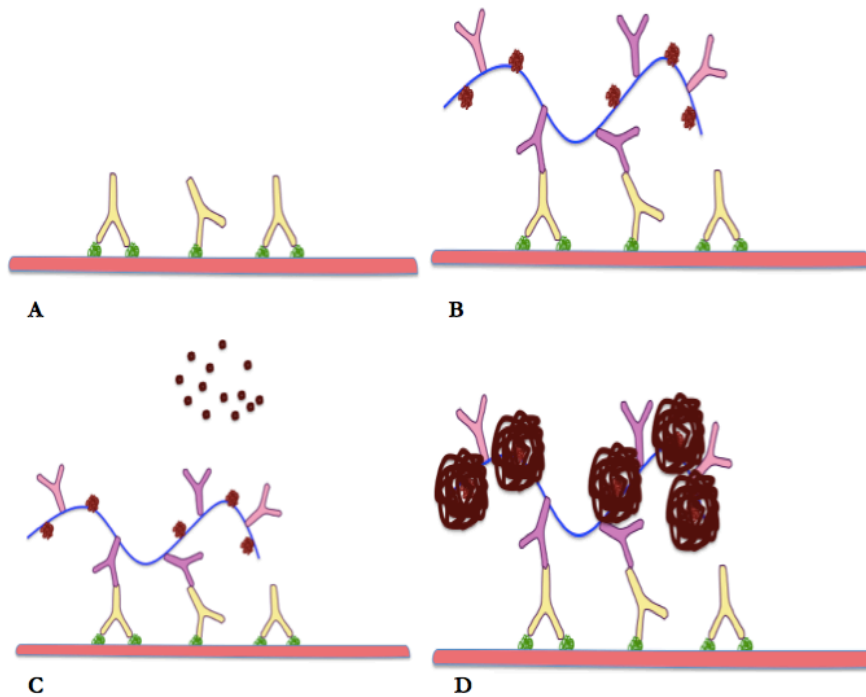
Figure XV A-D. Principal steps of immunohistochemistry. A) Monoclonal antibodies from mice (yellow) binds to a galectin antigen (green). B) Large polymers with secondary antibodies (rabbit, anti-mice; purple) together with an enzyme (HRP) are added. C) A substrate (diaminobenzidin; brown dots) is added. D) The substrate binds in to the enzyme (HRP), resulting in a brown-black staining of the specimen, showing the presence and location of the galectin molecule.

Statistical Considerations

Mainly non-parametric methods were employed throughout this thesis. Values are presented as median (range) and means (standard deviation). Continous variables were analysed using the Mann-Whitney *U* test, Kruskal-Wallis one-way ANOVA (with post hoc Scheffé test). Categorical data were analysed with Fischer´s exact test and χ^2 test. Regression analysis (univariable and stepwise) was used to identify significant predictors (I, III). Confidence intervals for proportions were calculated using normal approximation, and kappa statistics were performed to measure interobserver variability in the grading of dysplasia (II). *P*<0.05 was considered statistically significant. The softwares used for calculations were Statview® version 5.0.1 (SAS Institute, Berkeley, California, USA) (I), SAS for Windows (SAS Institute Inc., Cary, NC, USA) version 9.3 (II) and SPSS for Windows software (Chicago, IL, USA) version 19 (III) and 20 (III).

Ethical Considerations

The Regional Ethical Board at University of Gothenburg (www.epn.se) and the Regional Committee for Research Ethics in South Eastern Norway (III) approved the studies.

RESULTS AND COMMENTS

Long-term IPAA Function in Patients with Different IPAA Designs (I)

Median follow-up time was 13 (1-22) years. Significant differences were obtained between K-pouch/hand-sewn (*KH)* and J-pouch/hand-sewn (*JH)*, as well as between K-pouch/stapled (*KS)* and J-pouch stapled (*JS;* Fig XVI). A regression analysis showed that age at surgery and pouch design were significant predictors of the Öresland score ($p<0.001$).
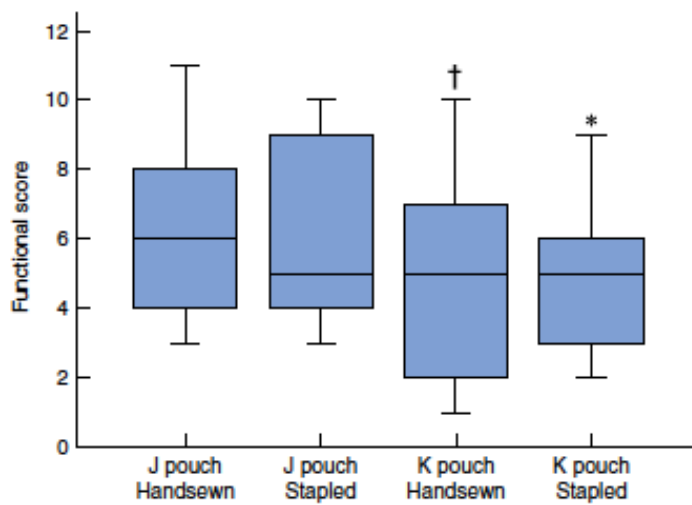
Figure XVI. Summarized functional score for included patients. Box plot. *JH* (n=96) mean 6.1, *JS* (27) 6.0, *KH* (95) 5.1, *KS* (194) 4.8. Horizontal lines within boxes, boxes and errors bars represent median values, interquartile ranges and 10th to 90th percentiles respectively. * $p=0.002$, + $p=0.034$ *versus JH*; * $p=0.047$ *versus JS*.

Although the Öresland score is supposed to be a summarized score and not an individual score, interesting differences were noted between the groups. Evacuation difficulties and use of retarding drugs were significantly more common among patients with *JS*. Moreover, 2/3 of the patients with *JH* suffered from soiling at night; only 1/4 of the patients with *KS* reported this

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problem. Use of protective pads was most common in the *JH* group. Compared to patients with *KS*, significantly more patients with *JH* had scores ≥ 8.

Comments

The obvious weakness of this study is that it is non-randomised. However, follow-up time of all groups is long; furthermore, relatively few surgeons performed all four combinations of pouches and anastomoses during the study period. The response rate is high (87%) and a substantial number of patients are included. The *K*-pouch with stapled anastomosis gives the best long-term functional outcome. However, the clinical relevance is not clear.

IPAA function is a complex parameter (see Introduction). Surgeons can probably affect the functional outcome by performing meticulous surgery, with nerve-sparing technique. Furthermore, two technical aspects have impact on functional outcome: 1. Design of the ileal pouch 2. Method for the ileo-anal anastomosis. A meta-analysis assessing outcome for *J-*, *S-* and *W*-pouches in 1500 patients revealed higher bowel movement frequency, and more use of retarding drugs and protective pads in patients with *J*-pouches compared to both **S**- and *W*-pouch[56]. However, the *S*-pouch was associated with need for anal intubation[56]. Few studies have been done on functional outcome in the long-term perspective. The majority of studies report a stable function over time[36,129].

Neoplasia in the IPAA (II)

The Ileal Pouch:

One of 56 (1.8%; 95%-CI: 0-5.3%) patients showed LGD in the pouch recorded by one pathologist. Each pathologist found IFD in 19 versus 20 patients, respectively. There was full agreement in 45 (80%) patients (14 with IFD and 31 without dysplasia; kappa=0.58). The majority of the patients with IFD demonstrated subtotal-total mucosal adaptation, moderate-severe chronic inflammation, and a score ≥3 with regards to acute inflammation (Table 5). No HGD or carcinoma was found.

Table 5. IFD in the pouch and grade of mucosal adaptation, chronic and acute inflammation.
Values are given as n and (%).

Mucosal adaptation	Pathologist 1 IFD/n (%)	Pathologist 2 IFD/n (%)
1	5/19 (26)	1/14 (7)
2	1/15 (7)	4/17 (24)
3	13/22 (59)	15/25 (60)
Chronic inflammation	**Pathologist 1** **IFD/n (%)**	**Pathologist 2** **IFD/n (%)**
1	0/8 (0)	1/16 (6)
2	7/26 (27)	5/21 (24)
3	12/22 (55)	14/19 (74)
Acute inflammation score	**Pathologist 1** **IFD/n (%)**	**Pathologist 2** **IFD/n (%)**
0	0/4 (0)	0/1 (0)
1	0/12 (0)	0/3 (0)
2	5/22 (22)	7/27 (26)
3	10/13 (77)	10/19 (52)
4	4/5 (80)	3/5 (60)
5	0/0 (0)	0/0 (0)
6	0/0 (0)	0 (0)

The ATZ:

29/32 patients with stapled anastomosis were analyzed. Remaining 3 patients lacked rectal mucosa in their biopsies. None of the pathologists found LGD, HGD or carcinoma. Pathologist 1 recorded two patients with IFD, pathologist 2 recorded four (kappa=0.31). The majority of the patients with IFD had moderate or severe inflammation. See Table 6.

Table 6. IFD and grade of chronic inflammation in the ATZ.
Values are given as n and (%).

Chronic inflammation	Pathologist 1 IFD/n (%)	Pathologist 2 IFD/n (%)
1	0/17 (0)	1/20 (5)
2	1/6 (17)	1/5 (20)
3	1/6 (17)	2/4 (50)

Comments

The Ileal Pouch

In a review of the literature including 2040 patients from 23 studies, the pooled prevalence of dysplasia was 1,13 (0-18,75)%[270]. A nationwide study from Holland reported a cumulative incidence of pouch neoplasia of 3.7% after 15 years and 6.9% after 20 years[73]. The large study from Cleveland Clinic reported that 0.6% of the patients operated on for chronic colitis was recorded with pouch neoplasia; however, 3.2% after 10 years was recorded for patients operated on for dysplasia or carcinoma[72]. The cumulative incidence of neoplasia was 2.2% for patients without prior colorectal carcinoma and 29.5% for patients with prior colorectal carcinoma[72]. Previous reports of dysplasia in ileal pouches in patients with neoplasia in their proctocolectomy specimen (studies from 2000) are displayed in Table 7. In the studies by Kariv[72] and Derikx[73], patients identified postoperatively cannot be separatley identifed.

Table 7. Cases with dysplasia in the ileal pouch in patients with previous neoplasia.

Reference	No	Duration IBD, y	Duration follow-up, y	Dysplasia [n]	Prevalence
Kupier 2008[168]	44	3-23 med 13	3-14 med 9	1 LGD/HGD	2.2%
Kariv 2010[72]	492	NA	NA	2 dysplasia	0.4%
Derikx 2013[73]	163	NA	NA	4 LGD	2.5%
Block 2014	**56**	**8-57 med 31**	**1-29 med 18**	**1 LGD/0***	**1.8%/0***

y, years; NA, not available; med, median; mo, months; post-op, postoperative
* Two independent pathologists

The ATZ

Previous reports of dysplasia in patients with neoplasia in their proctocolectomy specimen in the ATZ (studies from 2000) are displayed in Table 8. The results of the current study are in accordance with previous ones.

Table 8. Cases with dysplasia in the ATZ in patients with previous neoplasia.

Reference	No	Duration IBD, y	Duration follow-up, y	Dysplasia	Prevalence
Kupier 2008[168]	44	3-23 med 13	3-14 med 9	1 IFD	2.2%
Kariv 2010[72]	492	NA	NA	16 dysplasia	3.3%
Derikx 2013[73]	163	NA	NA	0	0
Block 2014	**56**	**8-57 med 31**	**1-29 med 18**	**0**	**0**

y, years; NA, not available; med, median

The clinical significance of IFD remains to be elucidated. Some studies include IFD in their analysis[168,270], while others do not[72,73]. IFD is not consider as a premalignant lesion during ordinary UC surveillance (colonoscopy) with a very low risk of progression to neoplasia[271]. Liu et al recommended that IFD in the pouch should be treated with antibiotics or anti-inflammatory treatment and followed by pouchoscopy every 3-6 months. However, the authors also stated that the meaning and impact of IFD is unclear.

Differences Between Patients with UC-PSC and Patients with UC-only (III)

1. Indications for Colectomy

Indications for surgery were different between the groups. In patients with IPAA, dysplasia was the indication for surgery in 23% in patients with UC-PSC vs 5% in UC-only. In the patients with IRA, corresponding figures were 41% vs 4%. Sixteen percent of the patients with UC-PSC and IPAA had carcinoma as indication for surgery, corresponding data for patients with IRA was 12%. Three percent of the patients with UC-only and IPAA (and no patient with IRA) had carcinoma as indication for surgery.

Comments
The results are in accordance with previous ones[171,202]. It is well known that indications for colectomy differs for patients with UC-PSC compared to patients with UC-only[171,202].

2. Functional Outcome after IPAA or IRA

For *IPAA*, there was no difference between patients with UC-PSC and patients with UC-only. For patients with UC-PSC, 6 (25%) of the patients had a score ≥ 8 compared to 7 (12%) in patients with UC-only.

For patients with *IRA*, the score was significantly worse for patients with UC-PSC compared to patients with UC-only (see Table 9). In patients with UC-PSC 2 (25%) had a score ≥ 8, compared to 3 (8%) with UC-only.

Comments

IPAA: There are only two previous studies on IPAA function in patients with UC-PSC[205,211]. Kartheuser et al[205] reported a mean daytime stool frequency of 6 (range 1-10), similar to that of patients with UC-only. Also in the other study, (65 patients with UC-PSC compared to 260 patients with UC-only), functional outcome was similar between the groups[211].
As recorded by others[206,208-210], patients with UC-PSC had significant more episodes of pouchitis (see below). Pouchitis is regularly associated with deteriorated functional outcome[102,272]. However, no such effect was observed in the present study.

IRA: The number of patients with UC-PSC and IRA in the functional analysis was small. However, patients with UC-PSC had significant worse function than patients with UC-only, see Table 9. The functional score for the patients with UC-only are roughly in accordance with previous reports[40,52].

No previous study has explored this topic. Considering the significant differences (bowel movement frequency and urgency), the most obvious explanation for the impaired function in patients with UC-PSC would be a higher frequency of proctitis[41,51]. Surprisingly, we did not find an obvious correlation between proctitis and functional outcome (see below, Procitis).

Table 9. Functional outcome IRA.

Feature	Rating	UC-PSC	UC-only
		IRA n=8	IRA n=39
Bowel movements [n (%)] during daytime			
≤4	0	2 (25)	12 (31)
5	1	0	20 (51)
≥6	2	6 (75)	7 (18)*
Bowel movements [n (%)] during night-time			
0	0	0	20 (51)
1/week	1	3 (38)	13 (33)
≥2/night	2	5 (62)	6 (15)**
Urgency [n (%)]	1	4 (50)	1 (3)***
Social handicap [n (%)]	1	4 (50)	2 (5)****
Median score (range)		7 (2–11)	3 (0–11)#
≥8		2 (25)	3 (8)

*p=0.003, ** p=0.014, *** p=0.0018, **** p=0.005, # p=0.005

3. Pouchitis

Details of pouchitis are displayed in Table 10. Significantly more patients with UC-PSC had experienced pouchitis compared to patients with UC-only. Furthermore, four episodes of pouchitis or more, was more common in patients with UC-PSC. Patients with UC-PSC had shorter time to pouchitis and more patients experienced their first episode within one year.

Table 10. Clinicopathological characteristics of pouchitis among the groups.

Pouchitis	UC-PSC n=31	UC-only n=62
Patients with pouchitis [n (%)]	27 (87)	20 (32)*
No episodes [n (%)]	4 (13)	42 (68)*
1–3 episodes [n (%)]	7 (26)	12 (60)
≥4 episodes [n (%)]	20 (74)	8 (40)**
Time to pouchitis, years [median (range)]	1 (1–11)	3.4 (0.5–16)
First episode within 1 year of surgery [n (%)]	17/26 (65%)	6/20 (30%)
Failure due to pouchitis [n (%)]	3 (11)	0

* $p<0.001$, ** $p=0.001$

Comments

Six previous studies have explored pouchitis in patients with UC-PSC (Table 11).

The current results support the theory that patients with UC-PSC have an increased risk for pouchitis. Considering previous studies, choosed method for diagnosis was solely clinical in one study[212], and based on histology in two[210,211]. Clinical criteria was combined with histology in two studies[208,209], the method was not stated in one[205]. Three studies found that PSC was associated with chronic pouchitis[208-210], while two did not[211,212]. One study was uncontrolled[205]. Most studies suggest that pouchitis is more common in patients with PSC, however not all.

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Table 11. Pouchitis in patients with PSC – previous studies.

Study	% of pouchitis	Definition of chronic pouchitis	Follow-up
Kartheuser 1993[213]	50%	Not stated	71 months
Penna 1996[208]	63% in PSC 32% in controls Chronic 60% vs 15%	>2 episodes + antibiotic>15 days per month	Mean 62.5 months (12-144)
Aitola 1998[209]	90% in PSC 30% in controls Chronic 70% vs 11%	Either 6 episodes per year or need for suppressive medical therapy	Median 64 months (12-102)
Gorgun 2005[211]	14% in PSC 12% in controls	1) 4 episodes/year 2) symptoms 4 weeks 3) chronic therapy	68±50 months (UC-PSC) 102±62 months (UC-only)
Lepistö 2008[210]	48% in PSC 26% in controls	Not stated	Median 9 years (3-14)
Hoda 2008[212]	PSC not assoc. to chronic pouchitis	3 episodes per year and antibiotic/antiinflam	Not clear
Block 2013	**87% in PSC 32% in control Chronic 74% vs 40%**	**>4 times/year**	**Median 11 years (0-26) UC-PSC Median 14 years (1-25) UC-only**

4. Proctitis

There was a correlation between the Mayo score and the histopathological score, but no obvious correlation between the Mayo score and the Öresland score. The three patients with the lowest Mayo scores (0, 0 and 1) had Öresland scores 2, 7 and 11, while the three patients with the highest Mayo scores (5, 6 and 6) had Öresland scores 2, 6 and 8.

Comments

There are no previous studies on the association between rectal function and proctitis (evaluated by endoscopy) in patients with UC-IRA.

5. Surgical Complications

There were no significant differences between patients with UC-PSC and patients with UC-only. For patients with IPAA, the most common complications were stricture of the ileo-anal anastomosis, followed by small bowel obstruction. For patients with IRA, the most common complications were anastomotic dehiscence and small bowel obstruction.

Comments

Patients with IPAA:
A few studies (mainly retrospective) have recorded surgical complications after IPAA in patients with UC-PSC. Mathis et al[206] found a complication rate of 39% after 6 years. In the case-control study (UC-PSC vs UC-only) by Gorgun et al[211], a complication rate of around 50% was found in both groups; however, pelvic sepsis was more frequent in patients with UC-PSC (14% vs 5%)[211]. Kartheuser et al[213] concluded that patients with UC-PSC have more long-term complications; the rate was 55%[213] (compared with their previous results in patients with UC-only of 29%)[273]. The authors found it of outmost importance that liver function is stable at time of surgery[213]. The current study demonstrates a complication rate of around 50% after 10 years, with no difference between patients with UC-PSC and patients with UC-only. The results are thus similar to the ones found by Mathis and Gorgun[206,211].
Although the literature is sparse, it cannot be concluded that patients with UC-PSC suffer more frequently from post-operative complications, than patients with UC-only.

Patients with IRA
The complication rate for patients with IRA and UC-only is in accordance with previous reports[40,52]. No previous study has reported outcome after IRA for patients with UC-PSC. Although the number of patients is small, there was seemingly a trend towards a higher complication rate in this group.

6. Failure

For patients with IPAA, there was no difference in failure rate between patients with UC-PSC and patients with UC-only.

For patients with IRA however, a significant difference was recorded (Table 12).

Table 12. Failure IPAA/IRA.

Failure	IPAA		IRA	
	UC-PSC	UC-only	UC-PSC	UC-only
No. of patients with failure [n (%)]	5/31 (16)	4/62 (6)	9/17 (53)	11/51 (22)*

*p=0.03

Comments

Failure after *IPAA* is a well-known problem (see Introduction). The failure rate in patients with UC-only is in accordance with previous reports[35,36,129]. No previous study has explored the failure rate in patients with UC-PSC. Interestingly, the increased rate of pouchitis in patients with UC-PSC was seemingly not associated with more failures.

For patients with *IRA*, the failure rate in patients with UC-only fits well with previous findings[40,41,52,54]. As, for IPAA, no previous study has explored this topic in patients with UC-PSC. The outcome of IRA in patients with UC-PSC is disappointing, but they should be interpreted with caution since the number of patients is low. However, IRA should probably be considered as a temporary solution in this group.

Galectin Variations in Ulcerative Colitis (IV)

Galectin-1 was not expressed in colon epithelial cells or in inflammatory cells.

Galectin-2 was strongly expressed in the entire cytoplasm of colon epithelial cells in controls without any individual variation. In the UC-colon, the expression was identical in patients with mild and moderate inflammation. Patients with severe inflammation showed a reduced to minimal expression. Around half of the inflammatory cells showed a weak expression and almost all strongly positive cells were macrophages.

Galectin-3 was strongly expressed in control tissue, showing a typical gradient with strong surface epithelial expression. The same gradient was observed in UC-colon. We observed a diminished expression in some patients with mild and

moderate inflammation but the majority with severe inflammation showed a similar expression as the control group. A strong expression was seen in very few of the inflammatory cells and 10-15% showed a weak expression. The majority of positive cells were macrophages.

Galectin-4 showed a different pattern of staining with a typical supra-nuclear distribution, both in controls and UC. The expression was strong in the control tissue. We observed a decreased galectin expression with increased inflammatory activity. However, there was a great inter-individual variation. Galectin-4 was not present in inflammatory cells.

Galectin expression related to inflammation grade. In conclusion, we did not find any clear correlation between the galectin expression and the severity of inflammation among the UC patients. Instead, we observed heterogenic patterns among the majority of patients.

Comments

Previous studies have implicated galectins in patients with IBD as potential markers of disease as well as potential theurapeutic agents. The majority of previous data is from experimental animal models[235,240,274,275] and thus the clinical significance in the human situation is uncertain. Published clinical studies are limited regarding the number of patients included and their clinical status as well as their pharmacological treatment[250,251]. The method of choice to evaluate galectin expression has been immunohistochemical semi quantitative grading of immunohistochemistry[215]. However, this approach still needs to be improved in terms of standardization of immunohistochemical procedures (different available antibodies) and the staining evaluation (objective and adapted scoring system)[215]. Comparing results from different studies are therefore difficult. Alternatively, galectins can be analyzed with qualitative analysis, i.e. mRNA reverse transcription techniques[228], Western blot[253] and/or flourescence activated cell sorter (FACS)[250,253].

We analysed the entire intestinal wall wich gave the ability to evalute large tissue areas, illustrating the focality of the disease (see Fig. 2 in paper IV). Therefore, interpretation of small biopsies should be evaluated carefully and their representativity can be questioned. Furthermore, with increased amount of inflammation, the cell loss increases and therefore changes in the galectin expression can solely be due to loss of intestinal epithelial cells.

GENERAL DISCUSSION

The Optimal IPAA – Technical Aspects

IPAA function is a complex parameter depending on several factors and it affects the patients QoL[145]. The surgeon can improve the functional outcome in performing meticulous surgery, avoiding complications, and by constructing the pouch and the ileo-anal anastomosis in an optimal fashion. Furthermore, technical aspects can have impact on future surveillance (see below).

This thesis shows that the *K*-pouch is associated with better long-term functional outcome than the *J*-pouch, and that the stapled anastomosis is associated with better long-term functional outcome than the hand-sewn. Although the difference is significant, the clinical relevance is unclear. It would be a bold statement to recommend the international surgical community to perform *K*-pouches as standard procedure. Compared to the *J*-pouch, the *K*-pouch is technically more complex to perform. Introduction of the procedure could be associated with complications during the learning curve. However, in an unit that regularly performs CI:s, it is an excellent way to keep up the competence for the CI procedure, since IPAA with *K*-design is constructed in the same way as a CI (omitting the nipple segment).

It is well known that the stapled ileo-anal anastomosis has better functional outcome than the hand-sewn[67,68,136]. The stapled anastomosis is easier to construct, associated with less postoperative complications[67] and is easier to survey (see below). Some authors recommend that a mucosectomy and hand-sewn anastomosis should be performed when dysplasia is indication for surgery, especially when dysplasia is present in the lower third of the rectum[70]. However, several studies have shown that mucosectomy do not seem to be completely protective against rectal remnants; thus, there could still be a risk of dysplasia. Accordingly, the anastomosis of choice should be the stapled one. However, if the stapled anastomosis fails due to technical problems, or if the disease (Familial Adenomatous Polyposis or neoplasia) is localized to the very distal rectum, knowledge of how to perform mucosectomy and hand-sewn anastomosis is mandatory.

Surveillance of Neoplasia in Patients with IPAA?

Surveillance of neoplasia in patients with IPAA is a delicate issue with several aspects to consider.

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The Association Between Dysplasia and Carcinoma

General surveillance of patients with IPAA without increased risk for neoplasia does not seem indicated. The number of patients with IPAA worldwide is constantly increasing and the cases with carcinoma are few. Carcinomas are probably never going to be the aim for a surveillance program; such a program has to focus on patients with increased risk for dysplasia. The problem with that strategy is that it is based on the assumption that dysplasia is a risk factor for carcinoma. Considering the ileal pouch, the association has to be demonstrated.

The Estimated Frequency of Dysplasia

Several studies have indicated that the risk for dysplasia in patients without risk factors is low. Patients with neoplasia in the proctocolectomy specimen are probably the only group where it seems rational to consider surveillance. The upper limit of the 95%-confidence interval for the frequency of dysplasia in the IPAA in the current study is around 5% and the study by Kariv[72] indicates that the risk could be even higher in patients with carcinoma in the specimen. The problem is the obvious discrepancy between the estimated frequencies of dysplasia and the observed numbers of patients with carcinomas related to IPAA:s.

Review of Specimen

It seems reasonable to suggest that patients with dysplasia in the proctocolectomy specimen could be considered for surveillance. Inclusion in the program is thus going to depend on the pathology report. In the current study, around 50% of the patients were identified postoperatively, during routine review of the specimen. If surveillance is suggested, the importance of the pathology report has to be emphasised. The routines for management of these specimens should be optimised and the quality increased.

Method for Surveillance - Pouchoscopy

Many patients experience pouchoscopy as a discomfort and evaluation of the ATZ is the major problem, especially in patients with hand-sewn anastomosis. Furthermore, since the majority of dysplasias and carcinomas are in the ATZ:s, it seems reasonable to have specific focus on this area[70,166,184]. Some authors recommend examination under anaesthesia[72]; however, in today health-care, this is not feasible. Furthermore, routine anaesthesia would considerably increase the risks associated with the program. There is a risk for bleeding and perforation when performing biopsies. Although the risk seems small, it should be considered, since the effects could have dramatic effects for the patient.

Discomfort and risks associated with pouchoscopy will probably effect patient compliance throughout the program.

Dysplastic lesions are hard to discover and can present in several ways. There are no obvious characteristic features of the lesions, and (the common) concurrent inflammation makes the task even more difficult. Thus, there is clearly a detection problem in routine endoscopy[72]. Chromo endoscopy has been evaluated but no increase in detected cases with dysplasia was found[168]. Polyps and lesions should be biopsied and polypectomy should be performed in lesions>1 cm, since neoplasia could be present in around 10% of these[276]. Interestingly, in case reports on pouch carcinomas, around one third of the patients lack visible lesions[72]. Therefore, blind biopsies are advocated.

Alternative techniques have been suggested for early detection of pouch neoplasia. These techniques include biopsy recordings of DNA abnormalities such as mutations of *p53*, *APC* genes, k-*ras*, aneuploidy and lost of heterozygosis[87,170,173,185,277]. Stool DNA tests have been used in screening for colorectal carcinoma and can be efficient in that setting[278]. However, these data cannot be applied on patients with IPAA.

Routine pouchoscopy is thus associated with several problems and cannot be considered a satisfactory method for surveillance. Improvement of the current technique and development of alternative ones are important future aspects.

Management of Patients with Dysplasia

There are established routines when a patient with UC is identified with LGD or HGD in the colon-rectum[159]:

a. Two experienced pathologists should review the biopsies.

b. Follow up visits should be performed more often when LGD is recorded.

Considering patients with IPAA, it seems reasonable to adopt these parts of the routines. Recording of HGD is usually considered indication for surgery. When HGD is present (in repeated biopsies) in the ATZ, resection should be suggested. Perhaps the most difficult case is a patient with HGD in the ileal pouch; it seems easy to suggest resection. However, the evidence for pouch excision in this situation is poor (and the patient is going to suffer deteriorated QoL). A thorough discussion with the patient is mandatory. The significance of IFD remains to be elucidated. Keeping with the guidelines for surveillance of colon-rectum in patients with UC, IFD is without significance in the ATZ[159]. However, when IFD appears in the ileal pouch, the literature is not consistent.

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According to Liu et al, the patient should be monitored more closely (3-6 months) and treated with anti-inflammatory drugs, or antibiotics[165]. Kupier et al., and Scarpa et al., also recommend closer survey[168,270]. In contrast, the two largest studies, Derikx et al[73] and Kariv et al[72] excluded IFD from their analysis. Since the evidence is poor, it seems reasonable to be on the safe side and suggest that IFD in the ileal pouch is managed as LGD.

In conclusion, surveillance or not in patients with IPAA is still a matter of debate. The proposed risk group (previous nepolasia) is small. However, prospected studies on the efficacy and costs seem more reasonable than routine implementation.

Management of the Patients with UC-PSC after Abdominal Colectomy

There are no randomized trials on the two major options (IPAA and IRA) after abdominal colectomy for patients with UC-only. Considering the much smaller group with UC-PSC, clinical decisions are thus going to rely on low evidence data. Another important aspect is that the complication rate is very high when colorectal surgery is performed in patients with concurrent liver cirrhosis; furthermore, significant postoperative mortality has been reported in this group[279].

After abdominal colectomy, IPAA seems to be the preferred option for patients with UC-PSC. The patient should be informed that most studies demonstrate an increased risk for pouchitis. However, there is no data favouring that the increased frequency of pouchitis is followed by an increased rate of pouch failure. Interestingly, the course of colitis in patients with UC-PSC is usually considered quiescent compared to patients with UC-only; the course of pouchitis may follow the same pattern. Aggressive treatment of pouchitis has been suggested[212]. Patients with UC-PSC and pouchitis have, in some studies, been considered as a risk group for dysplasia and thus suggested for surveillance. The data on IRA in patients with UC-PSC is sparse. In the current study IRA was associated with poor functional outcome high risk for failure. However, the study is small and there is an obvious risk for patient selection.

Finally, after abdominal colectomy, QoL should be evaluated in future studies on patients with UC-PSC.

CONCLUSIONS

- *K*-pouch with stapled anastomosis gives the best long-term functional outcome. The difference compared to other pouch designs is small and the study is non-randomised.

- The incidence of pouch neoplasia is low in patients with previous neoplasia in the colorectal specimen.

- The value of screening for neoplasia in patients with IPAA can be questioned and prospective studies are warranted.

- The outcome of IPAA in patients with UC-PSC is similar to outcome in patients with UC-only, except for an increased risk for pouchitis.

- IRA in patients with UC-PSC is associated with worse functional outcome and high failure rate. The study is small and there is a risk for patient selection.

- Galectin expression in patients with UC seems to be depending more on disease focality and individual variation, rather than tissue inflammation grade.

SUMMARY IN SWEDISH - SAMMANFATTNING PÅ SVENSKA

Bakgrund

Ulcerös Colit (UC) är en kronisk tarmsjukdom som kännetecknas av inflammation i slemhinnan inom kolon-rektum. Genesen är i huvudsak okänd och komplex; en samverkan mellan genetiska och immunolgiska faktorer, tarmfloran och en förändrad permeabilitet i tarmväggen är de mest troliga patofysiologiska mekanismerna. Incidensen i Sverige är ca 10-15/100 000 och år. Insjuknandet sker vanligtvis i 20-35 årsåldern och sjukdomen drabbar kvinnor i något högre omfattning (1.2:1). Den initiala behandlingen av UC är medicinsk, men i ca 30% av fallen krävs kirurgisk behandling. Den vanligaste operationsmetoden är proktokolektomi med anläggande av bäckenreservoar (IPAA), men ett annat alternativ är ileorektal anastomos (IRA).

Detta avhandlingsprojekt studerar långtidsresultaten efter operation med IPAA, fokuserat på reservoarkonstruktionens betydelse för det funktionella resultatet och risken för att utveckla dysplasi i IPAA:n. En speciell patientgrupp är den med UC i kombination med primär skleroserande kolangit (PSC; UC-PSC), i denna grupp har vi studerat utfallet av IPAA och IRA och jämfört med UC-patienter utan PSC (UC-only). De kliniska studierna har kompletterats med en experimentell studie, där uttrycket av galektiner (kolhydratbindande proteiner) analyserats i kolorektala preparat hämtade från patienter med UC.

Frågeställningar

Skiljer sig det funktionella resultatet sig åt mellan olika IPAA-konstruktioner? (I)

Hur hög är frekvensen dysplasi i IPAA:n bland patienter som hade dysplasi eller cancer i sin bortopererade tarm? (II)

Skiljer sig utfallet av operation med IPAA eller IRA mellan patienter med UC-PSC och patienter med UC-only? (III)

Finns det en korrelation mellan galektin-uttrycket och graden av inflammation hos patienter med UC? (IV)

Metod

I: Reservoaren kan konstrueras på olika sätt (*J* eller *K*); det samma gäller den ileo-anala anstomosen (staplad [*S*] eller handsydd [*H*]). Patienterna rekryterades från Svenska Kolitoperationsregistret (numera SwibReg-Swedish Inflammatory Bowel Disease Registry) och fick besvara frågeformulär för utvärdering av bäckenreservoarens funktion (Öresland score).

II: Ur samma register (I, ovan) identifierades patienter opererade med IPAA och med dysplasi eller cancer i sin bortopererade tarm. Patienterna erbjöds deltagande i studien (klinisk undersökning, endoskopi av bäckenreservoaren, samt biopsitagning). Biopsierna granskades av två oberoende patologer. Akut och kronisk inflammation, indefinite for dyspalsia (IFD), låggradig dysplasi (LGD), höggradig dysplasi (HGD) och cancer registrerades.

III: En kohort med patienter med UC-PSC jämfördes med patienter med UC-only från registret (ovan, I). Funktion (Öresland score), frekvenser av pouchit, komplikationer och failure analyserades.

IV: Patienter med operationskrävande UC inkluderades konsekutivt i en IBD-biobank; fullväggspreparat analyserades. Patienter med cancer eller funktionsrubbning fungerade som kontroller. Inflammationsgraden klassificerades som mild (cryptit), måttlig (abscess) eller svår (ulceration). Det immunohistokemiska galektin-1 till -4-uttrycket semikvantifierades okulärt från inget (0) till starkt (3).

Resultat och slutsatser

I: 412 patienter inkluderades; svarsfrekvensen var 87%. Signifikanta skillnader förelåg mellan *KH* och *JH* samt mellan *KS* och *JS/JH*. Regressionsanalys visade att reservoartyp och ålder vid operation hade betydelse för funktionen.

I detta icke-randomiserade, men stora patientmaterial, var IPAA-konstruktion med staplad K-reservoar förenad med det bästa funktionella långtidsresultat.

II: 56/90 (39 män) möjliga patienter inkluderades i studien. Den ena patologen fann 19 patienter med IFD, den andra 20, det var full samstämmighet i 45 (80%) av patienterna. En patolog fann LGD hos en patient i bäckenreservoaren (1.8% [95% CI: 0-5.3%]). Inget fall med HGD eller cancer hittades.

Frekvensen av dysplasi hos patienter med IPAA är låg, även i denna utpekade riskgrupp (tidigare dysplasi/cancer). Övervakningsprogram för den aktuella gruppen behöver utvärderas prospektivt.

III: 48 patienter (31 IPAA, 17 IRA) med UC-PSC jämfördes med 113 patiener med UC-only (62 IPAA, 51 IRA). Funktionen för IPAA-patienterna skiljde sig inte mellan grupperna. För patienterna med IRA var funktionen sämre i UC-PSC-gruppen. Pouchit var vanligare i UC-PSC-gruppen. Hos patienter med IRA var frekvensen failure 53% i UC-PSC-gruppen, jämfört med 22% bland patienter med UC-only.

Patienter med UC-PSC och IPAA har större risk för pouchit. Patienterna med IRA och UC-PSC är få och resultaten därför osäkra, men IRA förefaller vara ett alternativ associerat med dålig funktion och hög risk för failure.

IV: 22 konsekutiva patienter (8 män) med UC samt en kontrollgrupp (n=8), inkluderades i studien. Vi fann ingen säker korrelation mellan inflammationsgrad och galektinuttryck.

Uttrycket av de analyserade galektinerna uppvisar ingen tydlig korrelation med graden av klinisk eller histologisk inflammation. Variationen i galektin-uttrycket förefaller snarare vara ett uttryck för förlust av epiteliala celler och/eller individuella egenskaper hos den enskilda patienten.

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"It always seems impossible until it´s done"

Nelson Mandela

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APPENDIX (PAPER I-IV)