New concepts in ulcerative colitis

A thin line between medicine and surgery

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NEW CONCEPTS IN ULCERATIVE COLITIS

A Thin Line Between Medicine and Surgery

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General introduction and outline of the thesis

GENERAL INTRODUCTION

Clinical presentation

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) that is restricted to the colon and rectum. It has a relapsing and remitting character, which only involves the mucosa. Patients typically present with bloody diarrhoea, cramping and bowel movements up to 20 times a day.[1] The quality of life is impaired do to excessive fatigue, weight loss and in some cases faecal incontinence. Depending on the anatomical involvement, UC can be classified as having proctitis, left sided colitis and extensive colitis. In some patients, ileal inflammation, also classified as 'backwash ileitis' is seen which can be misdiagnosed as Crohn's colitis. Patients with distal colitis may also present with inflammation surrounding the appendiceal orifice without involvement of the cecum, which is also called a cecal patch. Furthermore, distal colitis may progress towards the proximal colon, whereas extensive UC may regress over time. This pattern is difficult to predict and may influence the prognosis and the necessity for colectomy.[2]

Epidemiology

UC is the most common form of IBD worldwide. In Northern Europe and North America, the incidence of UC is approximately 9-20 patients per 100.000 inhabitants per year. In the Netherlands, the incidence varies between 7-8 patients per 100.000 inhabitants per year.[3] Although Asian countries have shown the lowest incidence rates, there is an increasing trend due to westernization of lifestyle and industrialization[4] Environmental risk factors which are associated to IBD are smoking, diets high in fat and sugar, medication use, stress, and high socioeconomic status.[5]

Diagnosis

Knowledge about the extent and severity of the disease is essential to determine the optimal treatment strategy and predicting the patient's prognosis. Endoscopy, together with histology, is currently considered the gold standard for the evaluation of disease extent and activity in UC patients.[6] The ECCO guidelines recommend the use of the Montreal classification for defining the distribution of disease to describe the maximal proximal disease extent of inflammation seen at colonoscopy.[7] Several endoscopic scores have been developed to measure disease activity in UC patients and are often used in clinical trials. The Mayo score is the most commonly used activity index and is composed of four categories (stool frequency, bleeding, physician assessment, and endoscopic appearance).[8] The partial endoscopic subscore is shown in **figure 1**.

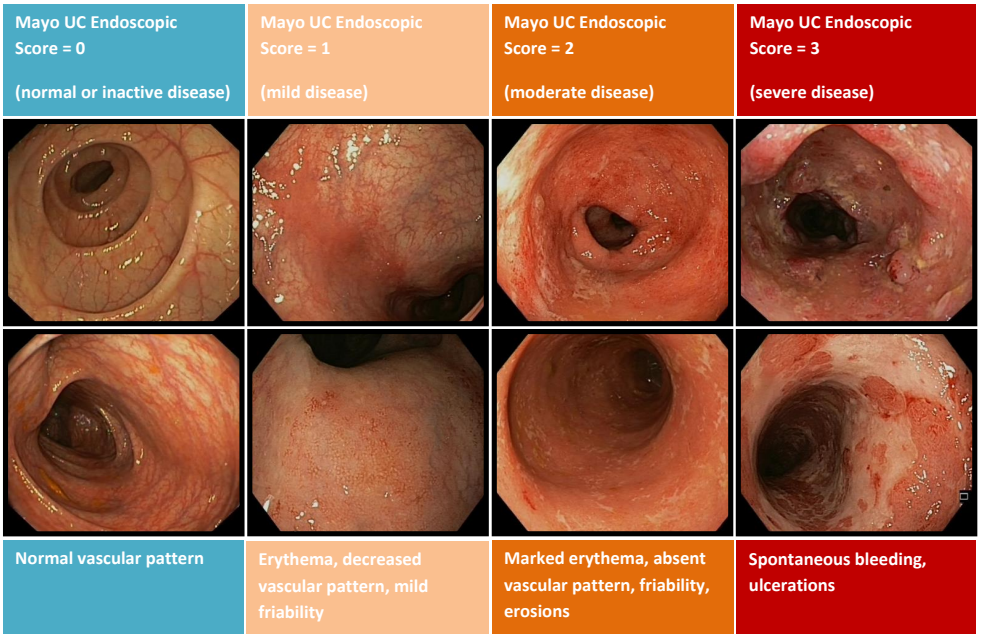

Figure 1. Mayo endoscopic subscore graded from 0 – 3

The most recent index for disease activity is the Ulcerative Colitis Endoscopic index of Severity (UCEIS), which is strongly correlated with patient reported symptoms and suitable for clinical trials.[9] Histologic evaluation can further differentiate UC from other forms of colitis. Inflammation can be graded according to the validated Geboes scoring system, which subdivides 6 grades based on structural (architectural) change: no abnormality, chronic inflammatory infiltrate, lamina propria neutrophils and eosinophils, neutrophils in epithelium, crypt destruction, and erosions or ulcerations.[10]

Medical treatment

Over the last two decades, medical treatment has evolved from suppression of symptoms, mainly with 5-aminosalicylic acid (5-ASA) and corticosteroids, to more immunosuppressive drugs and targeted therapies such as anti-TNF and integrin antibodies. According to the current guidelines; the treatment algorithm involves a step-up approach starting with the most conservative medication with the least risks and lowest costs.[11] However, the choice of treatment for patients with UC should be tailored to the level of disease activity (mild, moderate, severe), the extent of colonic involvement (proctitis, left-sided colitis, or pancolitis) and the course of the disease during follow up.[12] The treatment algorithm is depicted in **figure 2**.

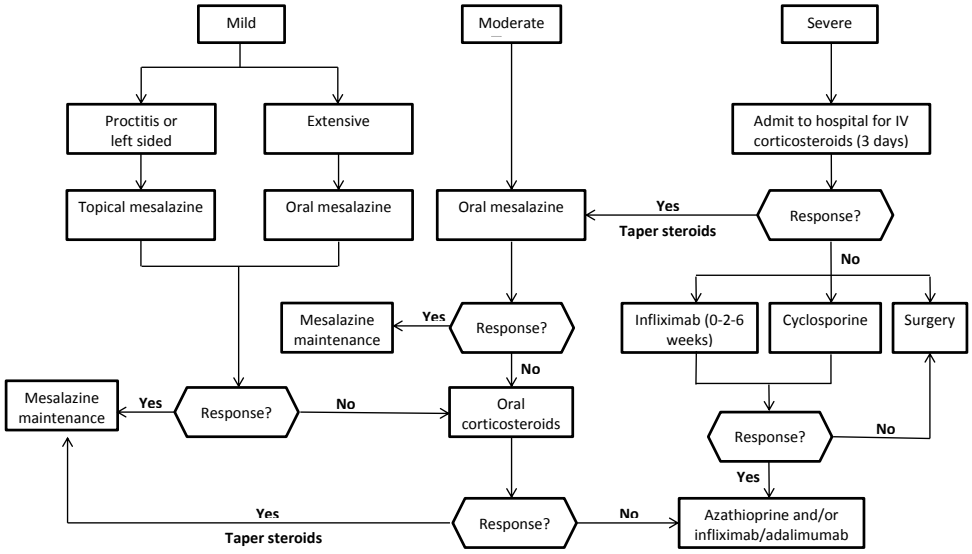

Figure 2. Treatment algorithm for ulcerative colitis

Surgery

Although medical therapy has advanced during the past decades, surgery continues to play an important role in the treatment of UC. Reported colectomy rates range from 5% to more than 20% at 10 years.[13,14] Patients with acute severe colitis or toxic mega colon are indicated for an emergency subtotal colectomy. Patients with therapy refractory colitis, steroid dependant colitis, or dysplasia/cancer can be treated in an elective setting. The timing of surgery is essential, ranging from hours to days for an emergency procedure and weeks to months for an elective procedure. Due to the dynamic nature of the disease, all treatment options should be discussed early in the disease course preferably in a multidisciplinary setting including both gastroenterologists and surgeons.

Ileal pouch anal anastomosis

Restorative proctocolectomy with ileal pouch anal anastomosis (IPAA) has become the standard surgical procedure in patients with UC, indeterminate colitis, familial adenomatous polyposis (FAP) and selected cases of Crohn's disease.[15] Significant improvements in surgical techniques and postoperative care have led to a reduction in postoperative complications. Nevertheless, anastomotic leak remains the most feared complication known as the Achilles' heal of pouch surgery, with an incidence that varies between 5-15%.[16,17] Furthermore, long-term complications such as small bowel obstruction, pouchitis and pouch failure have been described that may influence the quality of life.[18] In most practices, surgeons perform a multistage IPAA, involving the creation of a temporary defunctioning ileostomy as a protective measure for postoperative anastomotic leakage.[19] It has been suggested that a

defunctioning ileostomy avoids pressure on the fresh suture line, thus reducing the risk for leakage. The four different stages for IPAA construction are demonstrated in **figure 3**.

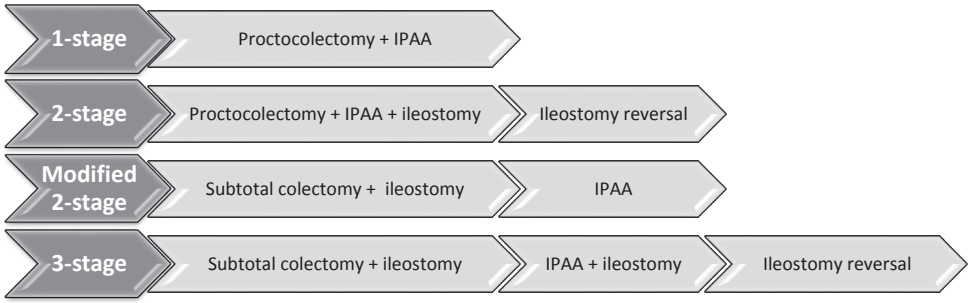

Figure 3. Stages restorative proctocolectomy with IPAA

Appendectomy

The appendix has been known as one of the last remaining controversies in medicine. This worm-like organ was named the vermiform appendix in 1530 by Vido Vidius and was later listed among the rudimentary organs of the human species by Charles Darwin and other proponents of the evolutionary theory (**Figure 4**). It was not until the late nineteenth century that it was acknowledged that most inflammatory diseases of the lower-right quadrant of the abdomen originate in the appendix. Mostly due to Charles McBurney's surgical work that described the clinical features of appendicitis, early surgical removal of the appendix became a commonly recommended procedure.[20]

Over the past few decades, a substantial body of evidence has accumulated supporting a role for the appendix in the development and course of UC. There is a strong inverse relationship between prior appendectomy during childhood and the development of UC.[21] In addition, a recent systematic review demonstrated that appendectomy might influence the disease course in UC patients, with possible reductions in relapse rates, need for immunosuppression and colectomy rates.[22]

A laparoscopic appendectomy is a relatively safe and simple procedure that can be performed in day care. If an appendectomy could play a therapeutic role in UC patients, this may decrease the need for life-long medication, including immunosuppression and biologicals and have a tremendous effect on the daily quality of life. Currently, two studies are being conducted to analyse the effect of an appendectomy on the clinical course of UC. A multicentre randomized controlled trial in which UC patients are treated until remission is achieved and subsequently randomized to either undergo appendectomy or standard medical treatment (ACCURE trial and ACCURE-UK trial, trial register; NTR2883) and the PASSION study, offering therapy refractory patients an appendectomy instead of a proctocolectomy with IPAA.[23]

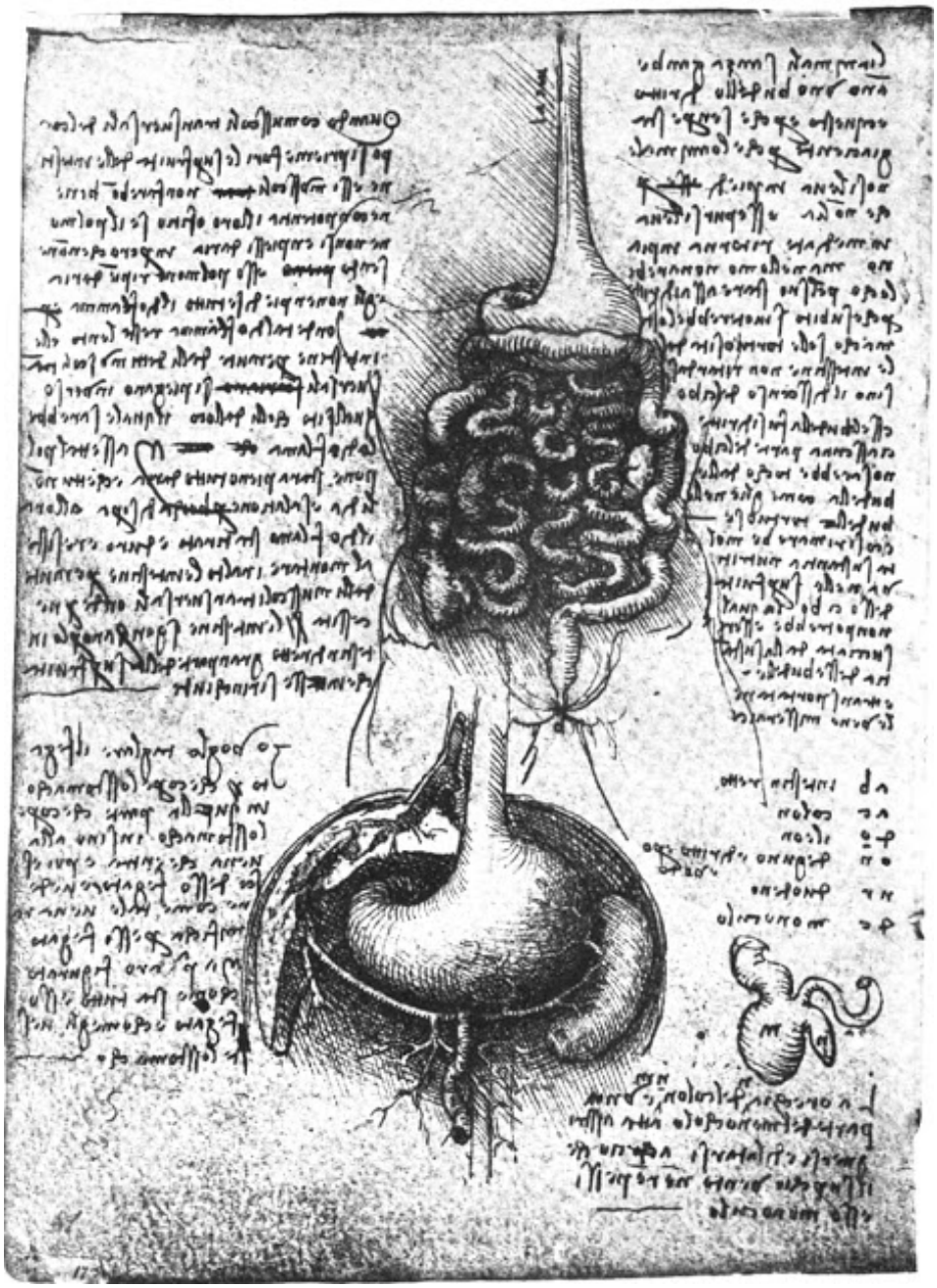

Figure 4. Anatomic drawing by Leonardo da Vinci, year 1504-6 (See right lower part of drawing: the cecum is marked with an M, the appendix is marked with an *N*)

OUTLINE OF THE THESIS

UC is a complex disease of which the exact aetiopathogenesis remains unresolved. While we've learned a great deal about the disease and have made significant advances, there are still pressing challenges in understanding the causes of UC, predicting the onset and course of disease and preventing complications once a patient is indicated for surgery. This thesis will therefore focus on the following research questions: what is the role of the appendix in IBD and could it be a priming site for UC. Has the natural history of UC changed over the past couple of years and is there a change in quality of life since the introduction of biologicals. Finally, which risk factors are associated to postoperative complications after pouch surgery and can we predict them in a validated model?

Part I: The effect of appendectomy in UC patients, experimental and clinical studies
The first part of this thesis focuses on the human vermiform appendix and sheds light on the immunological function and the association with UC. **Chapter 1 and 2** evaluates the current knowledge about the clinical and immunological aspects of the vermiform appendix in IBD and systematically reviews all epidemiological, cohort and case series. **Chapter 3** presents the short-term results of the PASSION study by examining the effect of an appendectomy in therapy refractory UC patients and analyses whether appendiceal pathological characteristics were predictive of clinical response. **Chapter 4** compares the immunological changes in the appendices of inflammatory and non-inflammatory specimens and describes whether appendiceal lymphocyte infiltration can be determined by analysis of lavage fluid.

Part II: Disease behaviour, quality of life and the risk of colectomy
The second part of this thesis explores the disease course of UC, quality of life and the risk of colectomy. **Chapter 5** demonstrates the changes in disease extension, disease behaviour and the risk of colectomy in UC patients in the era of biologicals. **Chapter 6** explores the health related quality of life and disability in a cohort of patients with moderate to severe UC who received anti-TNF treatment and patients that underwent restorative proctocolectomy with IPAA.

Part III: Restorative proctocolectomy and ileal pouch anal anastomosis
The third and final part of this thesis focuses on complications and functionality after restorative proctocolectomy with IPAA in both children and adults. **Chapter 7** demonstrates pre- and perioperative predictive **r**isk factors for anastomotic leaks after pouch surgery. **Chapter 8** evaluates the short and long term outcome of selective ileostomy formation in patients undergoing pouch surgery. A prognostic model of preoperative risk factors of pouch failure was externally validated in **Chapter 9**. **Chapter 10** demonstrates the long-term pouch

function in the paediatric and adult patient. **Chapter 11** reports the incidence and severity of pre-pouch Ileitis in comparison to pouchitis.

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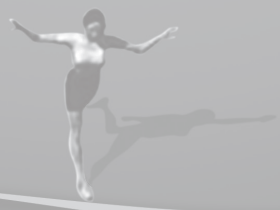
PART I

The effect of appendectomy in UC patients, experimental and clinical studies



CHAPTER 1

The link between the appendix and ulcerative colitis; clinical relevance and potential immunological mechanisms

S. Sahami, I.A. Kooij, S.L. Meijer, MD, G.R. Van den Brink, MD,
C.J. Buskens, A.A. te Velde

American Journal of Gastroenterology, February 2016

ABSTRACT

The human appendix has long been considered as a vestigial organ, an organ that has lost its function during evolution. In recent years, however, reports have emerged that link the appendix to numerous immunological functions in humans. Evidence has been presented for an important role of the appendix in maintaining intestinal health. This theory suggests that the appendix may be a reservoir or 'safe house' from which the commensal gut flora can rapidly be reestablished if it is eradicated from the colon. However, the appendix may also have a role in the development of inflammatory bowel disease (IBD). Several large epidemiological cohort studies have demonstrated the preventive effect of appendectomy on the development of ulcerative colitis, a finding that has been confirmed in murine colitis models. In addition, current studies are examining the possible therapeutic effect of an appendectomy to modulate disease course in patients with ulcerative colitis. This literature review assesses the current knowledge about the clinical and immunological aspects of the vermiform appendix in IBD and suggests that the idea of the appendix as a vestigial remnant should be discarded.

INTRODUCTION

Until recently, the vermiform appendix was mostly seen as a rudimentary part of the human intestine. However, awareness is increasing regarding the importance of this seemingly insignificant organ in the development and preservation of Gut Associated Lymphoid Tissue (GALT) and the interaction with intestinal flora.[1,2] With the growing number of reports linking the appendix to both the prevention and development of various pathologies (e.g. Clostridium difficile and ulcerative colitis (UC), a better understanding of its function and the role of the commensal gut flora on immunology might be helpful in daily clinical practice.[3–5]

This review aims to describe the current understanding regarding the immunological role of the vermiform appendix in both health and disease, and more specifically evaluating its role in UC. By studying its evolution and elucidating the ways in which it has changed, and more importantly features which have been preserved, a better understanding of its role in UC and the possible rationale of a therapeutic appendectomy will be provided.

THE EVOLUTIONARY PERSPECTIVE

Is the human appendix a vestigial organ?

In attempting to discover a plausible function of the human appendix, there has been a search for homology with that of other mammals. The appendix in humans is much smaller than in many other mammals such as rodents where the appendix serves a function in the fermentation of plant derived cellulose by forming a niche for specific cellulose degrading symbiotic bacteria.[6] This observation has resulted in the long standing view that the human appendix is a vestigial remnant, an organ that has lost its function during the evolution of species. However, more recent studies have argued that the appendix has evolved at least twice independently during evolution and that the presence of the appendix and appendix-like structures are in fact highly evolutionary conserved.[6,7] Indeed, although humans may not use the appendix to host cellulose degrading bacteria, it has been proposed that its worm-like morphology and location in the gut may function as a ‘safe house’ for the normal colonic flora. The appendix could be a reservoir from which normal microbial diversity could be rapidly restored after gastrointestinal infections.[7,8]

EVIDENCE FOR AN ASSOCIATION BETWEEN THE APPENDIX AND IBD

The effect of appendectomy on the risk of developing IBD

The first report suggesting a correlation between appendectomy and IBD was published in 1987. In this large case-control study, a significantly lower appendectomy rate was found in patients with UC compared to healthy controls, with reverse findings for Crohn's disease.[9] However, little attention was paid to this unexpected finding at the time. Only when other studies consistently confirmed a significant lower incidence of appendectomy in UC patients when compared to controls did it raise clinical interest.[10–12] Most subsequent studies confirmed this correlation. More recent studies draw attention to the fact that the decreased incidence of UC after appendectomy was predominantly seen in patients under the age of 20 years.[9,13–16] We performed a systematic search in Pubmed, Embase and Biosis to identify all published studies evaluating the role of an appendectomy in the prevention of IBD development. Thirty eight case control studies including a total of 15114 UC patients, have been published. The majority of these studies showed a significant inverse association between an appendectomy and the development of UC with an overall odds ratio of 0.39 (95% CI 0.29 – 0.52) **(Figure 1)**. The characteristics of all included studies are shown in the **Supplementary Table 1**. Obviously, the data should be interpreted with caution due to the retrospective character and small sample sizes of some studies. Furthermore, studies that have been published in the 90's did not always match their controls to the cases or control for confounding factors.

Apart from the case control studies, 4 large population based studies evaluated the incidence rate ratios of UC after appendectomy, of which 3 showed a significant lower UC incidence rate after appendectomized patients compared to control patients **(Table 1)**.[17–20] The largest, population based study, involving a total of 709,353 Swedish and Danish patients, addressed the question of whether the preventive effect of appendectomy is related to the underlying pathology, or whether it is merely caused by the operation itself. They demonstrated that, irrespective of familial predisposition, before the age of 20 years there was a significantly reduced risk of UC in patients who underwent an appendectomy for appendicitis (standardized incidence ratio 0.45, 95%CI 0.39-0.53), whereas an appendectomy without underlying inflammation was not associated with a reduced risk (standardized incidence ratio 1.04, 95% 95%CI 0.95-1.15).[21] The finding that an appendectomy may only be effective in case of appendicitis was rather new and confirmed in mouse studies.[22,23]

In Crohn's disease patients, the association with a previous appendectomy remains difficult to determine. In the same Scandinavian cohort described above, increased risk rates for Crohn's disease were shown, being highest within the first six months after appendectomy. However, these data are difficult to interpret since it may be affected by problems with the differential diagnosis between appendicitis and Crohn's disease that rather than a true association.[24]

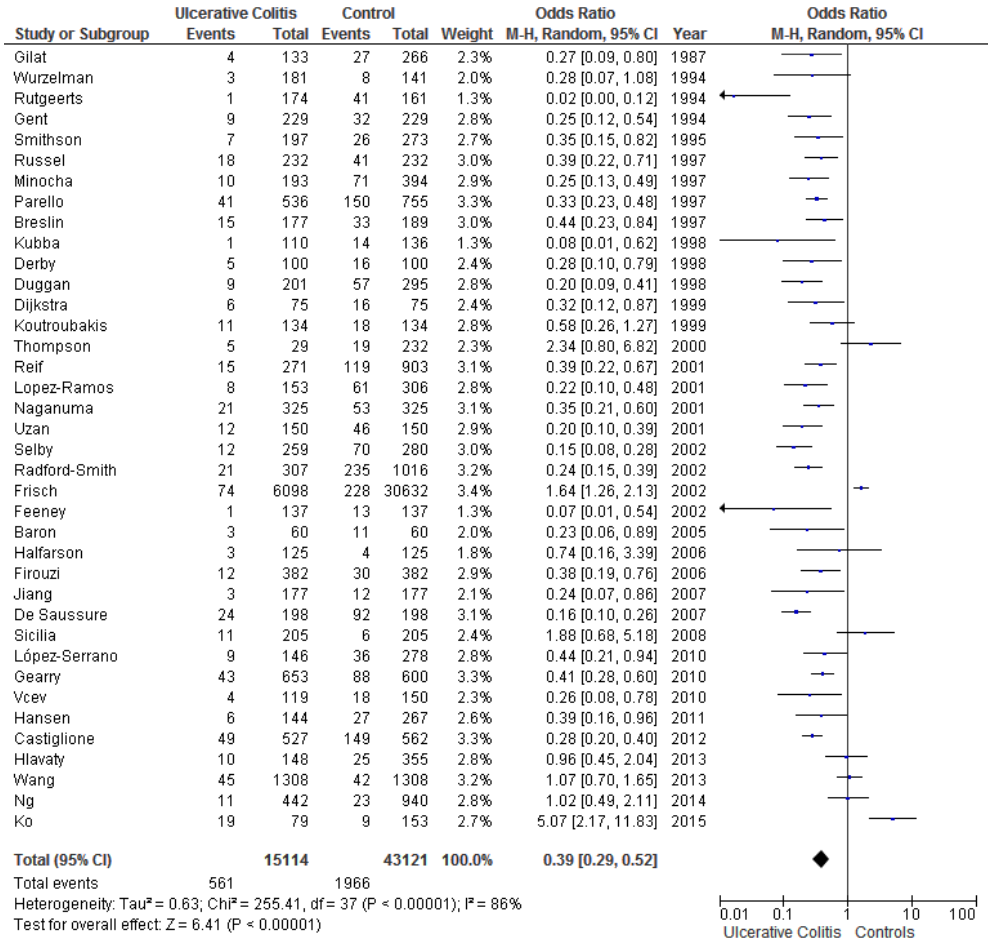


Figure 1. Forrest plot of all case control studies evaluating the effect of appendectomy on the risk of ulcerative colitis

Table 1. Studies evaluating the incidence rate of ulcerative colitis after appendectomy

Author	**Year**	**Country**	**Population** *Appendectomy vs Control*	**UC** *Appendectomy vs Control*	**Matched**	**Confounding factors**	**Incidence rate ratio** (95% CI)
Kurina	2002	UK	7273 – 7273	22 – 46[a]	Age and sex	No	0.48 (0.30 - 0.73)*
Hallas	2004	Denmark	234,559[b]	202	Age and sex	Age, sex, calendar year, pre- and post-appendectomy status	0.69 (0.57 - 0.85)
Frisch & Andersson	2009	Denmark & Sweden	709,353[b]	1192	Age and sex	Age, sex, calendar year	0.45 (0.39 - 0.53)*
Singhal	2010	UK	3829 – 3829	3 – 6	Age and sex	No	P = 0.34

[a] Observed vs expected UC rate
[b] No control group
*Incidence rate ratio for patients younger than 20 years

The effect of appendectomy on established disease

The potential role of the appendix in UC is underscored by a remarkable clinical observation. Peri-appendiceal inflammation can be observed in up to 70% of patients with proctitis or left sided colitis.[25–28] Indeed, the histological appearance of the appendix of patients with distal colitis can show the typical hallmarks of inflammatory bowel disease.[29,30] This very specific appendiceal inflammation that is observed in patients with disease that is otherwise restricted to the rectum or left side of the colon may suggest an ongoing role for the appendix in maintaining disease activity once the disease has been established.

However, there is only limited and conflicting data available regarding the therapeutic effect of appendectomy on the course of UC. Our recent systematic review identified six observational studies totaling 2532 IBD patients. Although a few studies found no effect, or even a detrimental effect, the majority of these studies showed a beneficial consequence of appendectomy.[31] Cosnes et al showed that previous appendectomy patients had a lower relapse rate and a decreased risk of colectomy. Interestingly, this effect was additive to that of current smoking and independent of other confounding factors for colectomy.[32] Although the study by Selby et al showed a negative association between an appendectomy and the development of UC, it failed to show any therapeutic effect on the disease course possibly due to a small sample size.[33] More recent studies found a moderate decline in hospital admission rates for disease relapse and a moderate decline in steroid use after appendectomy. [18,34] The authors argue that the decrease in disease activity may have been attributable to the natural course of UC, since the matched reference cohorts showed a similar decline. In our view, they did not account for other confounding factors such as disease extent and medication use and therefore remains questionable whether the groups were comparable in the first place.

The largest series by Bolin et al. showed excellent results in a prospective cohort of ulcerative proctitis patients. A total of 30 patients were treated, of which 90% showed a significant improvement in their clinical colitis activity index. In 40% of the patients there was a complete resolution of symptoms, which also resulted in withdrawal of all pharmacological treatments.[35] However, clearly none of the studies have been performed in a controlled manner, making the potential effect of appendectomy on the course of UC a matter of speculation that warrants well-designed clinical studies. The cohort studies evaluating the therapeutic effect of an appendectomy are shown in **Table 2**.

Table 2. Studies evaluating the therapeutic effect of appendectomy in ulcerative colitis

Study			Relapse			Requiring oral steroids			Requiring immunosuppressive therapy			Colectomy		
Author	**Year**	**Country**	**A+ (%)**	**A- (%)**	**P value**	**A+ (%)**	**A- (%)**	**P value**	**A+ (%)**	**A- (%)**	**P value**	**A+ (%)**	**A- (%)**	**P value**
Naganuma	2001	Japan	12/21 (57%)	239/304 (79%)	**0.031**	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cosnes	2002	France	47/98 (48%)	631/1024 (62%)	**<0.01**	33/47 (67%)	406/580 (70%)	**NS**	13/47 (27%)	110/580 (19%)	**NS**	8/49 (16%)	191/580 (33%)	**0.05**
Radford-Smith	2002	Australia	NA	NA	NA	NA	NA	NA	1/21 (4.8%)	71/286 (25%)	**0.04**	0/21 (0%)	60/281 (21%)	**0.02**
Selby	2002	Australia	NA	NA	NA	NA	NA	NA	4/12 (33.3%)	43/239 (18.0%|)	**NS**	2/12 (12.5%)	21/239 (8.8%)	**NS**
Selby[a]	2002	Australia	NA	NA	NA	NA	NA	NA	1/8 (12.5%)	43/239 (18.0%|)	**NS**	1/8 (12.5%)	21/239 (8.8%)	**NS**
Hallas[a]	2004	Denmark	0.53[bc]	0.51[bc]	**NS**	NA	NA	NA	NA	NA	NA	9/202 (4.5%)	42/808 (5.2%)	**NS**
Lee	2015	Korea	NA	NA	NA	40/68 (58.8%)	1427/2544 (56.1%)	**NS**	13/68 (19.1%)	500/2544 (19.7%)	**NS**	6/68 (8.8%)	207/2544 (8.1%)	**NS**
Lee[a]	2015	Korea	NA	NA	NA	0.19[c]	0.30[c]	**NS**	1.5[c]	1.0[c]	**NS**	0[c]	0[c]	NA

[a] Appendectomy performed during the course of UC
[b] Relapse measured as hospital admissions
[c] Before/after appendectomy ratio (and before/after reference date ratio in the non-appendectomy group)
NS: not significant
NA: Not available

The effect of appendectomy in mouse models of IBD

The effect of appendectomy on the development of colitis has been studied in various animal models. T-cell receptor (TCR)-alpha mutant mice spontaneously develop colitis with similarities to human UC. Intriguingly, appendectomy was strongly protective against the development of colitis in this model but only when performed at a young age of 3-5 weeks and was completely lost if the appendectomy was performed after 12 weeks of age.[36] The protective effect of appendectomy has also been observed in the dextran sulfate sodium (DSS) chemical model of colitis when mice were appendectomized at 6-8 weeks of age.[37] It has not been investigated if the effect of appendectomy was also age dependent in the DSS model. Finally, a protective effect of appendectomy was also observed in the commonly used T cell transfer model of colitis but the age at which the appendectomy was performed in these experiments was not mentioned.[38] In conclusion, three independent preclinical models support a role for the appendix in the development of colitis.

THE MECHANISTIC LINK BETWEEN THE APPENDIX AND UC

Despite the strong epidemiologic evidence of the protective effect of appendectomy in humans and the experimental evidence in mice, the molecular mechanism of the link between appendectomy and UC has not been resolved. Several theories have been proposed that involve both influence on the composition of the microbiota and homing of different immune cell populations, we will briefly review some of the most prominent theories below.

Dysbiosis and UC

In UC, a depletion of goblet cells and a defective inner mucin layer are observed to exist, leading to penetration of the luminal bacteria. It is uncertain whether these changes are causal, but it is tempting to speculate that the aberrant interaction with the gut flora contributes to the inflammatory response in UC.[39] Although dysbiosis is often seen in UC patients, this dysbiosis is variable between individuals.[40] Besides the possible causal relation with Fusobacterium varium species the bacteria that are strongly correlated with (the onset of) UC have not yet been distinguished.[41] Given the hypothesis that the appendix may be a reservoir for commensal bacteria, an appendectomy may be beneficiary in preventing re-colonization of the gut, which may in turn lead to amelioration of the disease course. In this sense, more research evaluating the microbial diversity in the appendix and UC, which may trigger the immunological cascade through the defective mucosal barrier, is needed.

IgA producing B cells

The appendix is part of the GALT and evidence in mice has shown that the appendix plays a critical role in the generation of IgA producing B cells that home to the colon but not the small intestine.[42] **(Figure 2)** Although the specificity of this mechanism for the colon is intriguing in the light of the specific association of the appendix with UC it is not entirely clear how reduced generation of colonic IgA producing B cells could predispose to IBD. The production of IgA is an important defence mechanism against pathogenic microbiota. In fact, recently it has been suggested that IgA specifically protects against a colitogenic microbiome.[43] Of course it could be that patients with UC have an aberrant repertoire of IgA producing B cells that in fact promotes a colitogenic microbiome. Such aberrant IgA repertoire could be hypothesized to underlie the development of colitogenic dysbiosis. if future studies convincingly demonstrate that this exists and actually plays a causative role in UC. However it should be stressed that such a scenario has not been examined to date.

NKT cells and the appendix

The appendix is a mucosal site that is very rich in so called Natural Killer T (NKT) cells. Although the precise role of NKT-cells in the pathogenesis of IBD is still elusive, increasing evidence suggest its involvement in the disease pathophysiology. A previous study has shown a correlation between UC and an aberrant Th2-type response, mediated by IL13-producing NKT cells. Experiments in the oxazolone mediated colitis model, which shows a Th2-like response and is believed to resemble UC demonstrated a key role for IL-13 signaling. IL-13 impairs the epithelial barrier function and therefore increases the exposure to luminal content **(Figure 2)**.[44] Compared to the colon and small intestine, NKT-cells are more abundant in the appendix, and tend to decrease with age. A recent study showed that contact with commensal microbes early in life may protect against NKT cell accumulation and thereby the risk of UC.[45] The correspondence with clinical data in which a beneficial effect of an appendectomy is linked to a young age, adds to the suspicion that NKT-cells play a role in the risk to develop of UC.[46] It should be pointed out however that a recent clinical trial in which IL-13 was blocked in patients with UC has been convincingly negative.[47] This study does not eliminate the possibility of a causal role of NKT-cells in UC that may not (solely) depend on the production of IL-13 but it does call into question the oxazolone mediated colitis model as a means to study the pathophysiology and therapy of human UC.

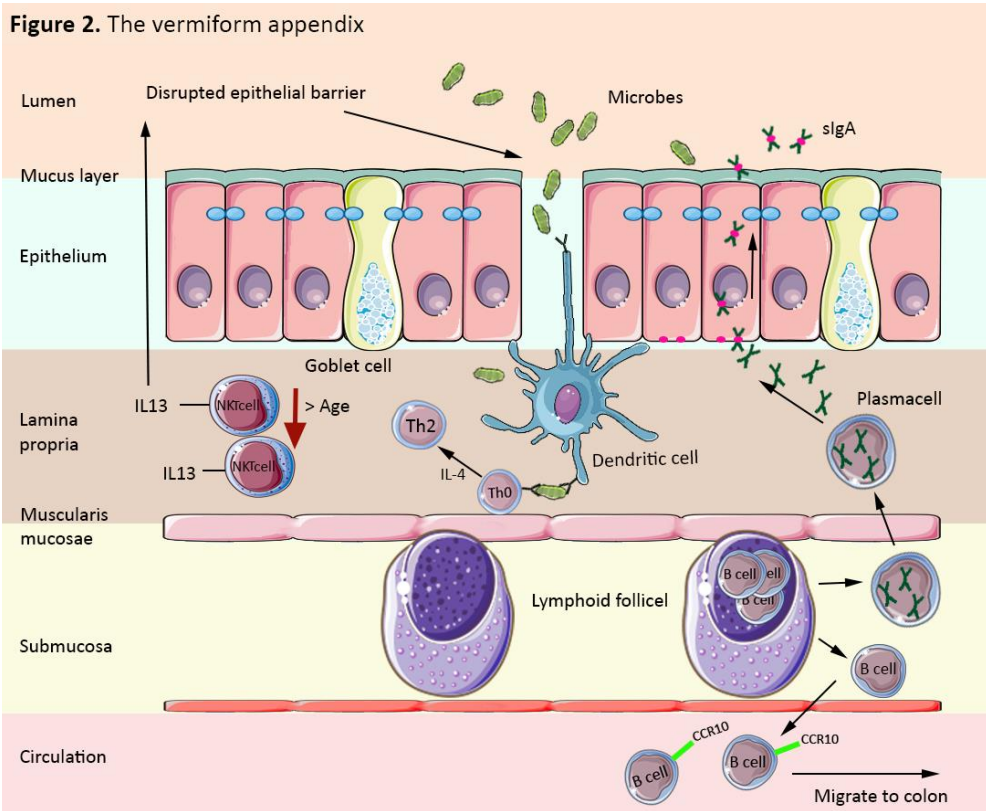


Figure 2. The mechanistic link between the appendix and ulcerative colitis. The left side of the image shows the Th2-type response, mediated by IL13 producing NKT-cells. The right side of the image shows the production of IgA producing B-cells by lymphoid follicles, which are abundantly located in the submucosa of the appendix. Lamina propria plasma B-cells produce a dimeric form of IgA cells (dIgA), which bind to receptors on the basal cell membrane and are carried out to the lumen as secretory IgA (sIgA). B-cells may also migrate to the colon mediated by the chemokine receptor CCR10. Appendectomy may inhibit these mechanisms.

FUTURE CHALLENGES

The appendiceal involvement in UC is now generating widespread interest. Although evidence is emerging suggesting a relation with UC, firm conclusions cannot yet be drawn. Many questions regarding the immunological function and its role in the pathogenesis of UC currently remain unanswered. The first and most important question is, whether there are significant beneficial effects of a therapeutic appendectomy on the disease course of UC patients. Currently, we are conducting a randomized controlled trial in which UC patients are treated until remission is achieved and subsequently randomized to either undergo

appendectomy or standard medical treatment (ACCURE trial and ACCURE-UK trial, trial register; NTR2883).[48]

Secondly, future research should focus on the significance of the microbiome and its diversity in the appendix. The contribution of microbiota in the pathophysiology of IBD is evident. However, research evaluating the microbiome of the appendix in UC is lacking. Further research should focus on identification of causal bacteria associated with UC within the appendix. Novel data concerning the interaction between appendiceal microbiota and the deranged mucosal immune system may have significant therapeutic implications by allowing the identification of potential new targets for drug development, or biomarkers for therapy response.

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Supplementary Table 1. Study characteristics of all case control studies evaluating the effect of appendectomy on the risk of ulcerative colitis

Author	Year	Country	Population *UC vs Control*	Appendectomy *UC vs Control*	Matched	Controlled for confounders	Outcome *Adjusted OR (95% CI)*
Gilat	1987	Israel Multicenter	133 – 266	4 – 27	Age, sex	No	0.27 (0.09 – 0.80)[a]*
Wurzelman	1994	USA	181 – 141	3 – 8	Age, sex, race	Smoking, childhood infections, antibiotics, IBD in family	0.30 (0.10 – 1.10)
Gent	1994	UK Multicenter	229 – 229	9 – 32	Age, sex	No	0.30 (0.10 – 0.60)
Rutgeerts	1994	Belgium	174 – 161	41 – 1	No	Age, sex, smoking	0.02 (0.00 – 0.12)[a]
Smithson	1995	UK	197 – 273	7 – 26[b]	No	Age and sex	0.20 (0.07 – 0.53)
Breslin	1997	Ireland	177 – 189	15 – 33[c]	No	Age, sex, social class	0.52 (0.24 – 1.12)
Minocha	1997	USA	193 – 394	34 – 20	No	Age, sex, race, smoking	0.87 (0.78 – 0.97)[a]
Parello	1997	Italy Multicenter	536 – 755	41 – 150	Age, sex, year of diagnosis	Smoking, tonsillectomy, alcohol, OCP	0.29 (0.19 – 0.48)
Russel	1997	Netherlands	232 – 232	18 – 41	Age, sex	Smoking	0.44 (0.24 – 0.78)
Derby	1998	UK	100 – 100	5 – 16	Age, sex, GP practice	Smoking	0.30 (0.10 – 1.00)
Duggan	1998	UK	201 – 295	9 – 57	Age, sex	No	0.14 (0.05 – 0.40)
Kubba	1998	UK	110 – 136	1 – 14	No	Sex	0.08 (0.01 – 0.62)[a]
Dijkstra	1999	New Zeeland	75 – 75	6 – 16	Age, sex, smoking	No	3.50 (1.15 – 10.6)
Koutroubakis	1999	Greece	134 – 134	11 – 18	Age, sex, education	Smoking, family history	0.38 (0.12 – 1.19)
Thompson	2000	UK Multicenter, Prospective	29 – 232	5 – 19	Sex, social class	No	2.34 (0.69 – 7.46)
Uzan	2001	France	150 – 150	12 – 46	Age, sex	Smoking, area of residence	0.26 (0.13 – 0.55)
Lopez-Ramos	2001	Spain	153 – 306	8 – 61	Age, sex	Smoking, tonsillectomy, OCP, education	0.23 (0.11 – 0.50)
Naganuma	2001	Japan Multicenter	325 – 325	21 – 53	Age, sex	No	0.36 (0.21 – 0.60)
Reif	2001	Israel Multicenter	271 – 903	15 – 119	Age, sex, Jewish group, education	No	0.39 (0.22 – 0.67)[a]
Feeney	2002	UK	137 – 137	1 – 13	Age, sex	No	0.05 (0.01 - 0.51)*
Frisch	2002	USA Prospective	6,098 – 30,632[d]	74 – 228	Age, race, first hospitalization	No	1.64 (1.26 – 2.13)

Radford-Smith	2002	Australia	307 – 1016	21 – 235	Age, sex	Age, sex, smoking	0.23 (0.14–0.38)
Selby	2002	Australia	259 – 280	12 – 70	Age, sex	No	0.15 (0.07 – 0.28)
Baron	2005	France	60 – 60	3 – 11	Age, sex, geographical location	Mother's educational level, family history of IBD, vaccinations	0.06 (0.01–0.36)*
Firouzi	2006	Iran	382 – 382	12 – 30	Age, sex	Smoking, tonsillectomy, NSAIDS, OCP	0.61 (0.29 – 1.28)
Halfvarson	2006	Denmark & Sweden	125 – 125	3 – 4	Twin pairs	No	0.5 (0.1 – 2.7)
De Saussure	2007	Switzerland	198 – 198	24 – 92	Age, sex	Age before 20 years, smoking, family history,	0.10 (0.05 - 0.21)*
Jiang	2007	China Multicenter	177 – 177	3 – 12	Age, sex	Smoking, alcohol, heavy tea intake, Capsicum consumption, family history	0.19 (0.05-0.78)
Sicilia	2008	Spain Prospective	205 – 205	11 – 6	Age, sex, rural /urban residence	No	NS
Vcev	2010	Croatia	119 – 150	4 – 18	Age, sex	No	<0.01
Gearry	2010	New Zeeland	653 – 600	NA	Age, sex	Many variables without correcting for multiple testing (city of residence, vaccination, antibiotic use, OCP, household, home healing)	0.41 (0.27 – 0.63)
López-Serrano	2010	Spain	146 – 278	9 – 36	Age, sex	Smoking, urban residence, parental occupation, post-secondary studies, respiratory tract infection, gastroenteritis as a child	0.17 (0.06-0.52)
Hansen	2011	Denmark	144 – 267	6 – 27	Age, sex, ethnicity, geographic location	No	0.29 (0.12 - 0.71)*
Castiglione	2012	Italy Multicenter, Prospective	527 – 562	49 – 149	No	No	0.28 (0.20 – 0.40)
Wang	2013	China Prospective	1308 – 1308	45 – 42	Age, sex	Smoking, breastfeeding, NSAID's, measles, parasites, OCP	0.929 (0.602-1.432)
Hlavaty	2013	Slovakia	148 – 355	10 – 25	Age, sex	Smoking, breastfed, sporting activities, number of siblings	1.02 (0.36 – 2.91)

Author	Year	Country	Population *UC vs Control*	Appendectomy *UC vs Control*	Matched	Controlled for confounders	Outcome *Adjusted OR (95% CI)*
Ng	2014	China Multicenter	442 – 940	NA	Age, sex, ethnicity, geographic location	Breast feeding, antibiotic use, pet animals, vaccination, childhood infections, sanitary conditions	1.021 (0.341 to 3.063)
Ko	2015	Australia Prospective	Migrants 79 – 153 Caucasian 77 – 173	NA	Age, sex	Family history, OCP, tonsillectomy, antibiotic use, pet ownership, sharing bedroom, pet feeding, takeaway consumption	Migrants 5.00 (1.59–15.70) Caucasian 0.47 (0.17 – 1.29)

[a] Recalculated OR and 95%CI from reported data
[b] Before (5) and after (2) onset of UC
[c] Before (11) and after (4) onset of UC
[d] Appendectomy in male adults (> 21 years)
* OR for patients younger than 20 years
NA: not available, NS: not significant, OCP: oral contraceptives

CHAPTER 2

The immunology of the vermiform appendix: a review of the literature

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SUMMARY

This literature review assesses the current knowledge about the immunological aspects of the vermiform appendix in health and disease. An essential part of its immunological function is the interaction with the intestinal bacteria, a trait shown to be preserved during its evolution. The existence of the appendiceal biofilm in particular has proved to have a beneficial effect for the entire gut. In assessing the influence of acute appendicitis and the importance of a normally functioning gut flora, however, multiple immunological aspects point towards the appendix as a priming site for ulcerative colitis. Describing the immunological and microbiotical changes in the appendix during acute and chronic inflammation of the appendix, this review suggests that this association becomes increasingly plausible. Sustained by the distinct composition of cells, molecules and microbiota, as well as by the ever more likely negative correlation between the appendix and ulcerative colitis, the idea of the appendix being a vestigial organ should therefore be discarded.

INTRODUCTION

Until recently, the human appendix has been regarded as a rudimentary part of the intestine. During the past few years, however, several studies have suggested its immunological importance for the development and preservation of the intestinal immune system [1]. The appendix has been shown to have an important interaction with the intestinal flora [2–4]. Considering the appendix as a 'safe house' for the commensal gut flora, these studies hypothesize that commensal bacteria can be reintroduced from the appendix in case of disease, and therefore the appendix can be considered as an important part of intestinal health. This literature review assesses the current knowledge concerning the immunological aspects of the vermiform appendix. By describing its normal physiology and the importance of its biofilm, and appraising its evolution and elucidating which aspects have changed or, more importantly, which have been preserved in the long history of its existence, a clearer understanding of its influence on the intestinal immune system will be provided.

The evolutionary perspective

In attempting to discover a plausible function of the human appendix, there has been a search for homology with that of other mammalians. The fact that the appendix is much larger in certain 'lower' mammalians such as rabbits has, for a long time, resulted in the human appendix being considered a vestigial organ. The lack of a clear morphological caecal appendage in some evolutionarily more closely related primates, however, seems to contradict this hypothesis [5].

In the assessment of its evolution, the human appendix is generally considered as a remnant of the mammalian caecum. Originally, this part of the intestine had a digestive function, primarily facilitating the digestion of cellulose with the aid of residential micro-organisms [6]. This cellulose digestive trait is lost in the human caecum, although in the human appendix a relative abundance of microorganisms present in biofilms still exists alongside the presence of lymphoid tissue [3]. The vermiform appendix in rabbits is found to be essential for the development of the gut associated lymphoid tissue (GALT). After the initial independent development of follicle centres, presence of the commensal intestinal flora is required for diversification of the primary antibody repertoire and for further development of T and B cell areas of follicles within the lymphoid tissue [7–9]. In some non-human primates, such as tamarins and white-eyelid mangabeys [5], and other mammals such as mice [10] and rats [11] that lack a caecal diverticulum, a high concentration of lymphoid tissue is found in the caecal apex [5], referred to as the 'caecal patch'. Moreover, the proximal large bowel of amphibians and reptiles, that lack both the presence of a caecum and appendix and the need of cellulose digestion, also functions as the site where most of the interaction between host and symbiotic bacteria is seen [12]. This gives rise to

the idea that the appendix, with its excellent conditions for sheltering the commensal gut flora, may have evolved prior to the caecum, rather than having derived from it. Therefore, the digestive trait could have been developed in conjunction with the bulging of the proximal large intestine that eventually became the caecum, which would imply that the immunological function existed before the digestive one. The worm-like morphology of the appendix and its location in the gut could indicate its long-lasting immunological function by providing a 'safe house' for the commensal intestinal flora, instead of being evidence for its vestigial nature [3,12].

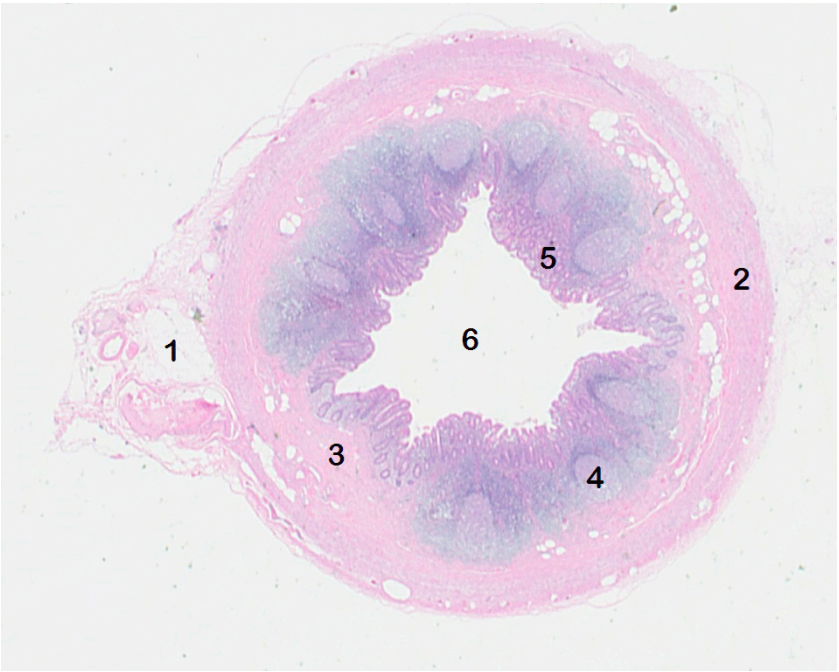

Figure 1. Transverse section of a healthy adult appendix. 1, mesoappendix; 2, muscularis externa; 3, submucosa; 4, lymphoid follicle; 5, mucosa and 6, lumen.

FUNCTIONAL HISTOLOGY OF THE APPENDIX

Similar to the intestinal wall of the colon, the appendiceal wall consists of a mucosa, submucosa, muscularis externa and serosa (**Figure 1**). Within these layers, however, the presence, quantity and function of cells differ between the appendix and colon, illustrated most notably by the presence of lymphoid follicles in the submucosa and lamina propria of the appendiceal wall [13]. The characteristics of cells and molecules found in human appendix are summarized in **Table 1**.

Table 1. Characteristics of cells and molecules found in human appendix

	Quantity compared to colon	Localization	Function
CD5[14,15] (B1-cells)	Increase	-	Production antimicrobial IgM antibodies and anti-self antibodies
CD19[14]	Increase	-	B-cell co-receptor
CD4CD69[16]	Increase during UC	Mucosa	Early activation of T-cells
CCL21[17]	Increase	Parafollicular areas	Attraction CCR7-expressing cells (T- ad B-lymphocytes and DCs)
FoxP3[18]	Increase during appendicitis	Lamina propria	Regulatory immune response
IELs[19-20]	Increase	(Dome) epithelium	Innate-like and adaptive immune response
a_4b_7[22]	Increase (b_7)	T-cells between lamina propria and epithelium, macrophages	Gut-homing
a_Eb_7[22]	Increase (b_7)	Mucosal $CD8^+$ T-cells, dendritic cells	Cellular retention in mucosa
IgG[1,23,24]	Increase	Lamina propria	Agglutination and opsonization of pathogens, complement activation
sIgA[4,25,26]	Increase	Mucosa	Agglutination of bacteria
Mucin[4,25,26]	Increase	Mucosa	-Formation intestinal mucus layers -Aiding formation biofilm by binding bacteria to mucus layer

Ig 5 immunoglobulin; UC 5 ulcerative colitis; DCs 5 dendritic cells; FoxP3 5 forkhead box protein 3.

Mucosa

The mucosa consists of columnar epithelium with enterocytes and goblet cells, a lamina propria and a muscularis mucosae. Next to macrophages, an abundance of immunoglobulin (Ig)A- or IgG- producing plasma cells is found in the lamina propria. Intraepithelial lymphocytes (IELs) in the appendix consist mainly of small CD81 regulatory T (Treg) cells [19], comparable with their presence in the epithelium of the colon. In the dome epithelium, also known as follicle-associated epithelium, which is located above the lymphoid follicles, the number of these IELs is increased to the rest of the appendiceal and colonic epithelium. Instead of only small Treg cells, M cells [27–29] and human leucocyte antigen D-related (HLA-DR) bearing Tand B cells [20] are also found here. As their presence is a sign of antigen transportation from the lumen and antigen presentation, respectively, it could indicate that the dome epithelium is an area of immune stimulation. Some of the IELs are morphologically similar to the cells in the follicle centre, giving rise to the speculation that IELs could, at least partly, have their origin in these follicles. Crypts of Lieberkuhn are present in the appendix, similar to the colon. Paneth cells, normally found in the small intestine, are found at the bottom of these crypts [30], with the production of anti-microbial peptides as their main function [31].

Submucosa

Submucosa consists of connective tissue and is characterized by the presence of many lymphoid follicles that extend from the submucosa into the lamina propria (**Figure 2**). While their presence or an equivalent structure is not seen in a healthy colon, they are comparable to Peyer's patches in the small intestine. The mantle zone of this lymphoid tissue, which is localized predominantly nearest to the lumen, contains densely packed B lymphocytes and few T lymphocytes. The dark zone within the distinct germinal centre is localized farthest from the lumen. It contains macrophages and centroblasts, proliferating B cells that give rise primarily to the follicle by monoclonal expansion. Centrocytes are derived from these centroblasts, and form the light zone together with follicular dendritic cells (FDCs) [32]. FDCs activate centrocytes by antigen presentation, which stimulates the production of immunoglobulins and prolongs their lifespan. Following CD40-CD40L interaction with T cells, centrocytes can also differentiate into plasmablasts or memory B cells [33,34]. Between the dome epithelium and lymphoid follicles another distinct area of immune cells is found: the mixed cell zone, consisting of macrophages and lymphocytes, B as well as T cells [20]. At the bottom of the lymphoid follicles are T cell areas, containing macrophages and T cells, with eightfold times more CD41 than CD81 T cells.

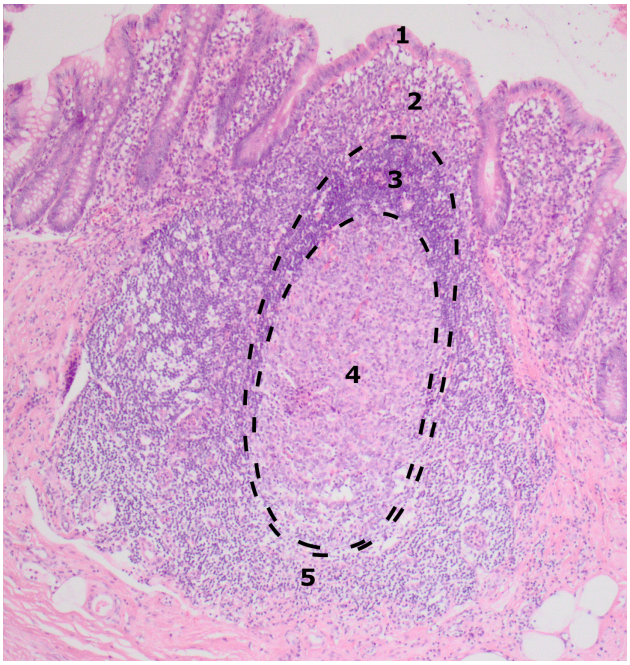

Figure 2. Appendiceal lymphoid follicle. Indicated are the distinctive areas of its most important constituents. 1, Dome epithelium: intraepithelial lymphocytes; 2, mixed cell zone: T-lymphocytes, B-lymphocytes, macrophages; 3, mantle zone: small B-lymphocytes; 4, Germinal centre: centroblasts, centrocytes, follicular dendritic cells, macrophages; 5, T-cell area: T-lymphocytes, macrophages.

LYMPHOCYTES IN THE APPENDICEAL TISSUE

The appendix has a distinct abundance of natural killer (NK)111 CD31 T cells (NK T lymphocytes), that can produce cytokines and chemokines rapidly following activation. Also the presence of B2201CD31 T cells, T cells expressing CD45R indicative for their activation, is increased compared to the rest of the gut [35]. A contributing factor to the abundance of lymphocytes may be the presence of CCL21, a chemokine present on the luminal surface of high endothelial venules and on lymphatic endothelial cells in parafollicular areas. By its binding to CCR7, CCL21 promotes the recruitment of B and T lymphocytes to the appendiceal lymphoid tissue and the migration of activated dendritic cells (DCs) back to lymph nodes [17,36].

Apart from the unique lymphocyte content of the appendix, difference is also found in molecules expressed on their surface when compared to the expression profile of intestinal lymphocytes. T cells in the lamina propria express more of the integrin subunit b7 compared to B cells in lamina propria and T and B cells throughout other parts of the gut. Integrin a4b7 is present primarily on T cells between lamina propria and epithelium, and on macrophages aEb7 mainly on mucosal CD81 T cells and DCs [22]. a4b7 binds to mucosal addressin cell adhesion molecule 1 (MAdCAM-1), with this interaction mediating the 'tethering and rolling' or 'homing' step in the attraction of lymphocytes. Because of the localization of its expression, a4b7 could therefore be regarded as a trafficking signal. Conversely, aEb7 is responsible for the retention of these lymphocytes, via binding with its ligand E-cadherin [37]. Intestinal DCs expressing aEb7 are believed to stimulate differentiation of forkhead box protein 3 (FoxP3)1 Treg cells after encountering (antigens of) bacteria. Logically, suppressing this differentiation into regulatory lymphocytes could lead to a proinflammatory state [21].

INTERACTION WITH MICROBIAL FLORA

The intestinal biofilm

The most luminal lining of the large intestinal wall contains the biofilm, a layer of commensal gut bacteria within a matrix of mucus that is believed to aid immune exclusion of pathogens by preventing them from crossing the intestinal barrier. The layer of thick, firm mucin that lays directly upon the intestinal epithelial cells is insoluble, thus preventing (pathogenic) bacteria from being in contact with the epithelium [38]. On top of this firm layer and directly adjacent to the lumen is a layer of looser mucin and commensal gut bacteria, together forming the biofilm [39,40]. In addition to the direct barrier function of the firm inner mucus layer, immune exclusion of potential pathogens could also be an indirect effect of the inclusion of microbiota in the biofilm, as bacteria within a biofilm are less likely to cross the epithelial barrier compared to single, planktonic bacteria [4,25].

Apart from mechanical barrier formation, biofilms shed bacteria actively from their surface. Shedding of planktonic bacteria could be seen as the mechanism behind immune exclusion of pathogens throughout the whole large intestine. Conversely, the shedding of parts of the biofilm itself is rather believed to facilitate (re)colonization of beneficial bacteria [41,42] (Fig. 3). This could be a function carried out exclusively by the appendix, as it is believed to be the only place within the large intestine that has not been cleared from its normal biofilm after diarrhoeal illness. During diarrhoea, turnover of enterocytes and thus shedding of the biofilm is accelerated [43], thereby leaving the intestinal wall derived of its protective barrier.

In contrast to the suggested induction of biofilm shedding by pathogens during acute diarrhoeal illness, diarrhoea-inducing infectious agents actually enhance mucin gene expression [44,45]. This enhancement, mediated particularly by cytokines such as tumour necrosis factor (TNF)-a [44], may indicate a stronger binding instead of an easier shedding. This could be seen as a reaction to the disruption of the mucus layers following bacterial invasion, but the definite function has not been determined. However, during an infection the intestinal wall shows an increase in goblet cells and mucus secretion compared to the healthy situation, which may explain the enhanced mucin gene expression. As this is associated with a better clearance of the pathogen [39], whether by speeding up the mucus turnover or creating a thicker layer, it could be seen as a defence mechanism against the infection.

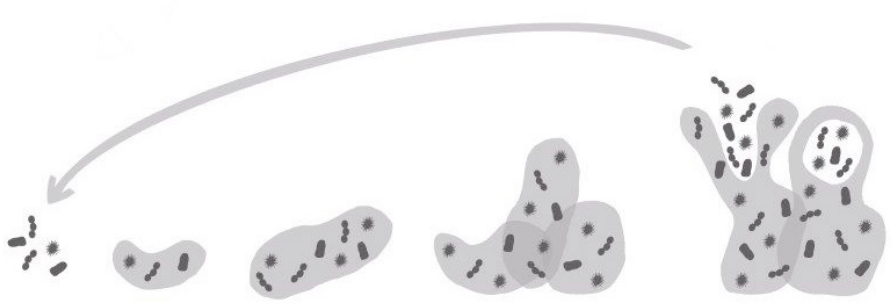

Figure 3. The process of biofilm formation, shedding and recolonization. Bacteria adhere to the surface. Biofilm formation and expansion by embedding bacteria within the mucin layer. Parts of the biofilm shed, which allows bacteria to relocate and recolonize (adapted from reference 42).

Special role for the appendiceal biofilm

The protected location in the most proximal part of the colon and its relatively little contact with faeces because of this location, and its narrow (worm-like) lumen, have given rise to the assumption that the appendiceal lumen is spared from the diarrhoeal clearance. Thus,

the biofilm in the appendix is thought to act as a 'safe house' for commensal bacteria and to facilitate their reinoculation of the gut after a gastrointestinal infection [2–4]. Secretory IgA (sIgA) and mucin assist in biofilm formation by increasing adhesive growth of the agglutinated gut flora [4,25,26], as sIgA stimulates the agglutination of bacteria and mucin binds these bacteria to the mucus layer. In the appendix, there is an overall high density of mucin and sIgA produced by B cells in the mucosa. Thus, the outer loose mucus layer of the appendix has a promicrobiotic environment, once again supporting its function as a 'safe house' [4]. Furthermore, the presence of commensal bacteria in neonatal intestines of mice causes an immune reaction by stimulating B cells in germinal centres to produce antibodies, thereby assuring a normal development of the immune system [46]. In humans, timing of the development of lymphoid follicles is consistent with the presence of bacteria in gut mucosa, both of which occur after the first 4 weeks postnatally [1].

The intestines of germ-free animals show a decrease in IELs, IgA levels and lamina propria lymphocytes [47], an impaired maturation of lymphocyte aggregates into isolated lymphoid follicles or Peyer's patches [48,49] and smaller germinal centres [49] caused possibly by the absence of proliferating B cells [50]. As the immune function within the intestine is otherwise impaired, it is therefore suggested that interaction with the commensal flora helps the GALT in developing an adequate immune response to pathogens [1,25,48,49]. Considering the high density of bacteria in the appendix and its assumed function as a 'safe house', it could indicate that the appendiceal biofilm has a crucial immunological role in aiding the development of a normal (intestinal) immune system.

CHARACTERISTIC APPENDICULAR CHANGES IN INFLAMMATORY BOWEL DISEASE

Acute appendicitis

Typical histological characteristics of acute appendiceal inflammation are mucosal ulceration [51], transmural infiltration of neutrophils and eventually perforation and serositis. In a more chronic stage of the appendiceal inflammation, infiltration of lymphocytes is observed [18,52]. Murine studies show an increase in the quantity of CD41 and CD81 T cells and a higher amount of FoxP31CD251 T cells in acutely inflamed appendices. FoxP31CD251 cells have a regulatory function, but they have been shown to be increased only in young mice and in the absence of antimicrobial substances such as antibiotics [18]. Regulation of inflammation occurs when FoxP31CD251 cells suppress IELs that are providing a protective function themselves, thus decreasing their production of cytokines and thereby tempering inflammation [21].

CD51 cells, also known as B1 lymphocytes, are found to a greater extent in healthy appendices compared to the rest of the gut, but even more so in inflamed specimens [14]. These CD51 B cells produce IgM antibodies against a broad range of pathogens. Antibody production takes place initially in the absence of antigen presentation by T cells, resembling an innate-like immune response such as that expressed by IELs [43]. Although these IgM antibodies have no high antigen affinity, they may still be important in first reaction to micro-organisms. Their increase could be explained by the simultaneous alteration in composition of the gut flora during acute appendicitis. Additionally, CD51 B cells also produce anti-self antibodies and the anti-inflammatory cytokine IL-10, thereby being of influence in autoimmune diseases [14,53].

Ulcerative colitis

In contrast to the transmural histological changes during appendicitis, a more chronic and autoimmune coordinated inflammation of the appendix is seen in ulcerative colitis (UC). A distinct increase in the occurrence of Paneth cell differentiation, goblet cell depletion and crypt abscesses is observed, while neutrophil infiltration is not prominent. This resembles colonic inflammation in UC rather than a 'normal' acute appendicitis, suggesting it to be a skip lesion of UC instead of a concurrent disease [54,55]. In fact, appendiceal orifice inflammation in UC is not exceptional, and is not seen solely in right-sided or pancolonic UC, but also in mild disease restricted otherwise to the left colon or rectum [56]. Additionally, in some cases an appendiceal orifice inflammation even precedes UC, suggesting that it may play a role in its development [57]. The appendiceal inflammation is confined predominantly to the mucosa and results in both a quantitative and qualitative change of the lymphocyte phenotype, with an increased ratio of CD41 to CD81 in T lymphocytes, because it is predominantly the early activation antigen CD69 in T lymphocytes [16,58] and the activation marker CD25 in both CD41 and CD81 T lymphocytes [54] that are increased in UC. This could indicate that the appendix acts as an early priming site for this particular disease that becomes activated before the rest of the colon.

Immunological link between acute appendicitis and ulcerative colitis

The pathway by which appendectomy seems to relatively protect patients against the development of UC [55,59,60] is not yet clear, but age at time of intervention [61] and use of antibiotics during treatment of acute appendicitis [62] have proved to be of influence. The protection against the development of UC by the combination of appendicitis and appendectomy suggests that characteristic immunological processes make the appendix a priming site for UC [16,58,63]. Possibly FoxP31CD251 T cells, CD51 and CD191 B cells play a special role, which will be elaborated further below.

The increase in regulatory FoxP31CD251 T cells during appendicitis could initiate a regulatory immune responseable to prevent autoreactivity, as is seen in UC. Age has proved to be the major limitation on the stimulation of FoxP31CD251 T cells in mice [18], just as the protective mechanism of appendectomy is achieved only when patients have undergone surgery before the age of 20 years. This similarity, that has not been found for other markers, makes it plausible that FoxP3 plays a certain role in the incitement of ulcerative colitis. The need for the absence of anti-microbial substances in order for FoxP31CD251 T cells to increase during appendicitis could indicate its crucial function in the intestinal response against bacteria. Their regulatory immune reaction could mean a lower to non-existing response to (commensal) bacteria in the colon, in contrast to the uncontrolled reaction that would normally take place at the incitement of UC [18]. Although data are not available for the appendix, the presence of regulatory T cells in colonic lamina propria during UC has been explored. One study shows a decrease of regulatory Tcells [64], which would be in line with the presumed defect in UC. Yet another demonstrates a contradictory increase in FoxP31CD251 T cells in colonic lamina propria during UC [65]. If this proved to be the same in the appendix, it would indicate that the increased amount of FoxP31CD251 T cells is not enough to regulate the ongoing inflammation. Nevertheless, it does not rule out the possibility of preventing UC before its onset.

CD51 B lymphocytes have been shown to be increased in acute appendicitis. In UC, however, the number of these cells is decreased, and with it also decreasing the quantity of natural antibodies and anti-self-antibodies. This could indicate that the presence of CD51 B cells has a protective role against UC. If so, it would only be expected that a period in life in which there is a relatively high concentration of CD51 lymphocytes, as is the case in acute appendicitis, could have a beneficial protective effect against UC [53].

The percentage of CD191 B cells is increased in healthy appendices compared to the rest of the gut, but this increase is even more profound in inflamed specimens [14]. CD19 is a common marker present on all B cells. It acts as a co-stimulatory molecule important for the survival, maturation and function of B cells [66]. The higher percentage of CD191 B lymphocytes, with their function in prosurvival signalling, could point towards the appendix being a site meant specifically for maturation and activation for B cells. Because the increase in CD19 during inflammation could actually be considered as an amplification of the already high preinflammatory status, this may even be an indication of the appendix being a priming site in UC.

MURINE STUDIES LINKING THE APPENDIX TO ULCERATIVE COLITIS

Various murine studies support the idea that the appendix may be of importance in the pathogenesis of UC. Despite the fact that mice lack a vermiform appendix, as mentioned earlier, they have a caecal patch that is considered the equivalent of the human appendix. A study analysing the migration of colitis-inducing CD62L1CD41 cells showed a 35-fold enhanced migration of these cells into the caecal patch compared to the colon. Inflammation in this model was more profound in the distal colon, suggesting that the appendix serves as a site to store and prime the colitis inducing CD62L1CD41 cells [67]. Furthermore, appendectomy (i.e. removal of the caecal patch) in several murine models with experimental colitis was shown to protect against the development of colitis. In experiments with T cell receptor-alpha mutant mice that develop inflammatory bowel disease (IBD) spontaneously, appendectomy at a young age (< 5 weeks) suppressed the development of colitis, whereas 80% of the sham-operated mice developed IBD [68]. In another study where colitis was induced with dextran sulphate sodium (DSS), mice that had undergone appendectomy showed a delayed onset of colitis and a milder disease activity [69]. These murine models, by describing the effect of appendectomy, could imply an important role for the appendix in the pathogenesis of UC.

INTESTINAL MUCUS LAYER AND MICROBIAL FLORA IN ULCERATIVE COLITIS

Both the inner firm and outer loose mucus layer discussed earlier are likely to play a role in the development of chronic intestinal inflammation. Mice with a deficient mucus layer show a different response when colitis is induced with DSS [70]. Defects in the inner layer, in particular, leading to penetration of the luminal bacteria, are thought to play a role in the initiation of UC [39]. The absence of MUC2, a subtype within the mucin family that is essential for the renewal and formation of the mucus layer, has been shown to lead to spontaneous inflammation [40] and to colon cancer in the long term [38,39], a process completely in accordance with the disease course of UC [71]. Whereas during acute (infectious) colitis an increase in goblet cells and mucus secretion is observed, correlating with a better clearance of the pathogen thus aiding recovery [39], the appendiceal goblet cell population is depleted in UC [54]. Whether this depletion and defective mucus layer are cause or effect of UC is still being questioned [72]. However, it would be interesting to speculate that it precedes aberrant interaction with the gut flora leading to the inflammation, because this would again present the appendix as a possible priming site, given its rich bacterial content.

Although dysbiosis is often seen during UC, there is no distinct composition of gut flora during UC [66], as the dysbiosis is variable in individuals [73]. Until now, no bacteria are

found that are correlated strongly with (the onset of) UC besides a suggested possible causal relation with Fusiform varium [74]. Studies examining the effect of antibiotic or probiotics in IBD patients [21,66,73,75,76] have inconsistent and disappointing results, which may be explained by this wide variation of gut flora composition in UC patients.

GENETIC AND ANTI-MICROBIAL INFLUENCES

It is stated that genetic susceptibility might play a role in triggering IBD, with a total of 200 IBD risk loci identified through genome-wide association studies [77,78]. As the increase in the incidence of IBD in the western world since the 19th century is too rapid to be attributed only to genetic risk factors, it is argued that genetic susceptibility is particularly relevant in combination with environmental factors [78,79]. It has been suggested that this observed trend may be explained by the hygiene hypothesis. In essence, the hygiene hypothesis argues that a lower infection rate is the underlying cause of the increasing incidence of autoimmune diseases such as UC because of the lack of protection against immunological disorders carried out by certain infectious agents [80]. Proponents reason that anti-infectious factors such as use of antibiotics, vaccinations and the relatively uncontaminated western diet can influence the intestinal microbiome. The lower exposure to pathogens causing this alteration could lead eventually to a dysbiosis which, in turn, could induce or perpetuate abnormal immune reactivity, as is seen in UC [81]. However, recent studies demonstrated no statistically significant association for most hygiene-related risk factors in residents of fully developed western countries [79,82], but discovered these factors to be still influential in migrants moving from low- to high-incidence countries [79]. The only consistent association among hygiene-related environmental factors is reported between antibiotic use in childhood and an increase in incidence of IBD [62,79,83]. This association could be explained by mechanisms described earlier: as appendicitis is believed to induce a regulatory immune response, anti-microbial treatment treating the inflammation would consequently interfere with this effect. Indeed, in mice suffering from acute appendicitis that were treated with antibiotics, the percentage of FoxP31 T-reg cells of the total CD81 cells was observed to be decreased to a nearly normal level. In mice without the anti-microbial treatment, this percentage was more than twice as high [18].

CONCLUSION

The vermiform appendix is not a rudimentary organ, but rather an important part of the immune system with a distinct function within the GALT different from lymphoid tissue in other parts of the intestine. Having examined the evolutionary characteristics, it can be deduced that the core function in origin lays in the interaction with and the handling of intestinal bacteria. It influences GALT by stimulating its development and aids recovery after diarrhoeal illness by recolonizing the colon with commensal flora.

The observations elaborated in this review support the idea that a defective function and interaction with gut flora in the appendix play an essential role in the aetiology and probably also in the onset of UC. However, it remains uncertain whether the dysbiosis seen in appendices of many UC patients is the cause or result of the inflammation. Based on this, it can be concluded that the appendix has an important immune function both in health and disease. There are, however, numerous interesting aspects left unstudied. Progress could be made in understanding the influence of the appendix on the development of GALT and on normally functioning gut flora. Further research should focus upon identification of causal bacteria associated with UC within the appendix, which might improve health care by earlier detection of the disease and amelioration of treatment.

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CHAPTER 3

Therapy refractory UC patients with mucosal appendicitis may benefit from appendectomy: the PASSION study

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Submitted

ABSTRACT

Objective: The objective of this study was to examine the effect of an appendectomy to modulate the disease course of therapy refractory UC patients, and analyse appendiceal pathological characteristics predictive of clinical and pathological response.

Methods: Patients with therapy refractory UC, and referred for proctocolectomy were invited to undergo laparoscopic appendectomy first. Results were measured by the Mayo score (partial clinical 0 – 9 and endoscopic 0 – 3). The primary endpoint was clinical response after 3 and 12 months. Clinical response was defined as a decrease in the partial Mayo of ≥3 points. Secondary endpoints were endoscopic remission, failure and pathologic response. Remission was defined as an endoscopic Mayo ≤1 point. Failure was defined as when patients underwent colectomy or started trial medication. Appendiceal resection specimens, and pre- and post-operative biopsies (or colectomy resection specimens in case of failure) were histologically graded according to the validated Geboes score (0-5; 0= no inflammation, 1-4= increasing grades of inflammation, 5= ulcerations and erosions). In addition, appendiceal resection specimens were stained for CD4 (grade I-IV) and sIgA:IgG ratio to characterise inflammatory characteristics immunohistochemically. Pathological response was defined as any decrease in the Geboes score of colonic biopsies postoperatively and correlated to clinical response. Factors predictive of pathological response were analysed by regression analysis.

Results: Thirty patients (53% male) with a median age of 40 (IQR, 33 – 47) underwent appendectomy with a median preoperative total Mayo score of 9 (IQR, 8-11). After 3 months, clinical response was seen in 17 (57%) patients of whom 7 (41%) were in remission. After 12 months, 9 (53%) had lasting clinical response of whom 5 (56%) were in remission. Three patients had a late clinical response. Eleven patients failed (7 colectomy, 4 trial medication). Pathological evaluation was possible in 22 patients. In 11 patients there was microscopic active inflammation of the appendix (Geboes grade 2-4). After a median 13.0 weeks (range 7-51), pathological response was seen in 11 patients (47.8%) with a median decrease of 2 points (range 1-3). There was no correlation between pathological and clinical response (p=1.0). Appendiceal inflammation was highly predictive of pathological response when compared to no inflammation or extensive ulcerations (8/10 versus 3/12, p=0.02). Immunohistochemistry showed that increased numbers of CD4+ T-lymphocytes and a decreased ratio of IgA:IgG in the appendix were predictive of pathological response, which was immunohistochemically characterized by an increased IgA:IgG ratio in colonic biopsies or resection specimens.

Conclusion: Appendectomy was effective in at least 30% of therapy refractory UC patients, with a substantial proportion of patients demonstrating complete endoscopic remission after 1 year. Pathological response was related to active inflammation in the appendix, while there appears to be a discrepancy between how patients feel and what is seen during colonoscopy and pathology. These early results suggest that UC patients with mucosal appendicitis may benefit from appendectomy. However, long-term follow up is warranted to exclude a possible placebo effect.

INTRODUCTION

Up to 30% of patients with ulcerative colitis (UC) ultimately require surgery.[1,2] These patients have been extensively treated medically (often including trial medication), and are refractory to biologicals or steroid dependant. Optimizing medical treatment for inflammatory bowel disease resulted in a decreased risk of surgery over the years, but this effect is most pronounced in Crohn's disease (CD)[3] Therapy-refractory UC patients are often in deplorable condition, preventing direct restorative proctocolectomy. The surgical treatment of choice is a colectomy with end-ileostomy, followed by completion proctectomy with ileal J-pouch anastomosis at a later stage.[4] Although the postoperative outcomes are satisfactory, it involves at least two major abdominal surgeries, and a more colon sparing approach would be preferred. Various studies have evaluated the effect of a laparoscopic appendectomy as a therapeutic option in UC patients. A recent systematic review identified six observational studies evaluating the effect of an appendectomy in patients with an established disease. The majority of studies demonstrated a beneficial consequence either in reducing the need for immunosuppressant, reducing the relapse rate or even the colectomy rate on the long term.[5] The largest case series by Bolin et al. of 30 ulcerative proctitis patients that underwent appendectomy demonstrated a significant improvement of the Simple Clinical Colitis Activity Index (SCCAI) in 90% of the patients of which 40% were able to withdrawal all pharmacological treatments.[6]

Although the pathogenesis of UC remains unresolved, it is often speculated that the defective mucosal barrier function and cytokine imbalance play an important role in the aetiology of the disease. Penetration of luminal bacteria trough the mucosal wall may cause an aberrant interaction with innate immune cells causing an inflammatory response. One of the theories that has been proposed linking the appendix to UC is that the appendix, with its inner layer of mucus and secretory IgA (called biofilm) functions as a reservoir for commensal bacteria. These bacteria can be secreted into the colon, influencing the microbiome and the immunological response. In this sense, an appendectomy may prevent re-colonization of the gut and lead to a milder disease course. Another theory is that the cytokine production within the appendix may trigger an immunological cascade in the colorectum causing inflammation, this may be prevented by an appendectomy.[7,8]

If a relatively safe and simple appendectomy could postpone or even prevent colectomy in therapy refractory UC patients, this will result in enormous advantages for these patients, with a substantial gain in health and reduction in costs. The objective of this study was to examine the effect of an appendectomy to modulate the disease course of therapy refractory UC, and analyse appendiceal pathological characteristics predictive of clinical response.

METHODS

Study design

This prospective pilot study was conducted in two tertiary IBD centres (Academic Medical Center, Amsterdam, the Netherlands and St. Vincent's Hospital, Dublin, Ireland) between August 2012 and December 2015. Patients over 18 years of age with therapy refractory or steroid dependent UC, requiring colectomy despite complete medical treatment were invited to undergo compassionate use laparoscopic appendectomy first. Patients were considered completely treated when 5-acetylsalicylic acid (5-ASA), corticosteroids, immunomodulators and biologicals (FDA approved) have been attempted with minimal to no results. Each patient was thoroughly discussed at the multidisciplinary meeting during which the indication for colectomy and inclusion for the study was discussed by IBD gastroenterologists and surgeons. Patients were excluded from the study in case they had a prior appendectomy or other abdominal surgery, if there was any suspicion of Crohn's disease, toxic mega-colon or other emergent surgical indication, patients with active extra-intestinal infections, liver or kidney failure, mayor lung and heart co-morbidity. Patients with insufficient command of Dutch or English or cognitively unable to complete questionnaires were also excluded from the study.

Patients underwent an ileocolonoscopy before appendectomy and after 3 and 12 months to assess disease activity and mucosal appearance. Biopsies were taken when possible. Patients signed an informed consent form approved by the institutional review board.

Surgical procedure

Laparoscopic appendectomy was performed within 6 weeks after inclusion. All appendectomies were performed by or under supervision of a dedicated IBD surgeon in day care setting. Care was taken not to touch the appendix during the surgical procedure to avoid influencing microscopic findings. The appendix and cecal base were resected using a laparoscopic endostapler enabling a safe and complete resection including the orifice of the appendix with the possible peri-appendicular red patch (PARP), also called a cecal patch. The resection specimen was handled according to a standard operating procedure and used for histological and immunohistochemical evaluation. Patients continued their medical treatment as given pre-appendectomy during the study period. After 3 months, patients were evaluated clinically and endoscopically, and in case of response medication was tapered.

Outcome measures

Results were measured by the Mayo score, which is the most commonly used activity score for UC (ref mayo). The total Mayo score is composed of four categories; three non-invasive

clinical categories (bleeding, stool frequency, physician assessment) and one objective category (endoscopic appearance). Each category is rated from 0-3 totalling a score that ranges from 0-12.[9] In this study we evaluated the (partial) clinical Mayo score and the endoscopic Mayo score separately. Primary endpoint was the clinical Mayo score after 3 and 12 months. Secondary endpoints were endoscopic remission, failure, and pathological response at 3 months. Clinical response was defined as a decrease in the clinical Mayo score of ≥3 points. Remission was defined as an endoscopic Mayo score ≤1 point. Failure was defined as when patients underwent colectomy or started trial medication (eg. Vedolizumab, Etrolizumab).

Pathologic assessment

Pre- and post-operative biopsies (or appendiceal resection specimens in case of failure) were histologically graded according to the Geboes score (0-5). The Geboes score is a validated pathology score for UC and subdivides 6 grades based on structural (architectural) change: no abnormality, chronic inflammatory infiltrate (architectural changes), lamina propria neutrophils and eosinophils, neutrophils in epithelium, crypt destruction, and erosions or ulcerations.(Geboes ref) Pathological response was defined as any decrease in the colonic Geboes score postoperatively, and correlated to clinical response. Although the Geboes score has been developed to grade colonic inflammation in UC, this score was also used to grade appendicular resection specimens in this study. Since it has been previously demonstrated that appendices in UC often show mucosal inflammation with comparable characteristics, this score was considered most appropriate to inventory inflammatory changes.

Immunohistochemistry

Appendicular paraffin embedded slides were stained for CD4 (Biolegend, San Diego, CA), as it has been previously demonstrated that the increased numbers of lymphocytes seen at histology were predominantly CD4+ T cells, and that influx of mucosal CD4+ T cells correlated to histological disease activity. Slides were scored according to the number of positive cells per high power field. A representative mucosal area was chosen which was not directly covering a lymphoid follicle in the submucosa or lamina propria of the appendiceal wall. Scores were adapted from Stumpf et al. (grade 1 representing no staining and grade 4 extensive lymphocyte infiltration).[10] Appendicular slides, and pre- and postoperative colonic slides, were also immunohistochemically stained for immunoglobulin-A (IgA) and immunoglobulin-G (IgG) (Biolegend, San Diego, CA).

Statistical analysis

Continuous data are presented as mean and standard deviation (SD) or as median and interquartile range (IQR) according to the distribution. Categorical data are presented as

frequencies and percentages. To compare dichotomous data the χ2-test or Fisher's exact test were used. Independent t-test was used to compare means. The dependent t-test was used for repeated measures. Mann-Whitney-U test was used for continuous, not normally distributed data. Factors predictive of clinical and pathological response were analysed by logistic regression analysis. The IgA and IgG results were presented as a ratio. All tests were analysed two-sided and a P-value of <0.05 was considered significant. Statistical analyses were performed using IBM® SPSS® for Windows® version 22 (IBM Corp., Armonk, NY, United States).

RESULTS

Demographics

In total, 30 consecutive patients (53% male) with a median age of 40 (IQR, 33 – 47) underwent laparoscopic appendectomy. In 6 (20%) patients the disease was limited to the rectum, in 12 (40%) patients there was left sided disease and in 12 (40%) patients there was extensive disease. Eight (27%) patients were treated with anti-TNF and 12 (40%) were treated with steroids. The median preoperative total Mayo score was 9 (IQR, 8-11)), with a partial clinical Mayo of 6 (IQR 5-8). Eleven (37%) patients showed an endoscopic Mayo score of 2 and 19 (63%) an endoscopic Mayo of 3 (**Table 1**). No major postoperative complications occurred. One patient had an itchy skin rash after anaesthesia, which resolved after one day. Another patient needed additional pain medication due to abdominal tenderness.

Table 1. Demographic data

	N=30
Male *(n, %)*	16 (53)
Age in years (*median*, *IQR)*	40 (33 – 47)
Disease extent *(n, %)*	
Proctitis	6 (20)
Left sided	12 (40)
Extended	12 (40)
Disease duration in years (*median, IQR)*	8 (3 – 14)
Medication *(n, %)*	
None	8 (27)
5-ASA	17 (57)
Immunomodulators	10 (33)
Anti-TNF	8 (27)
Steroids	12 (40)
Preop full Mayo score *(median, IQR)*	9 (8 – 11)
Preop partial clinical Mayo score (median, IQR)	6 (5 – 8)

Clinical response

After 3 months, 4 patients failed (3 colectomy, 1 trial medication). The postoperative Mayo score decreased significantly when compared to the baseline Mayo (total Mayo score 4.5 versus 9.0, p=0.001, partial clinical Mayo score 2 versus 6, p=0.001). Therefore, clinical response was seen in 17 (57%) patients of whom 7 (41%) were in remission (5 patients refused endoscopy at this time point). Nine patients showed minimal to no change in their symptoms.

After 12 months, of the remaining 26 patients, 7 failed (5 colectomy, 2 trial medication). In the 17 patients that showed initial clinical response, 9 (53%) had lasting clinical response of whom 5 (56%) were in remission (3 patients refused endoscopy). Three of the 9 patients that initially did not respond at 3 months, showed a late clinical response at 12 months (Figure 1). All patients were able to withdraw steroid treatment due to resolution of their symptoms. Two of the 8 patients that were on anti-TNF treatment continued their therapy despite resolution of their symptoms.

Pathologic evaluation of the appendix

Pathological evaluation, with comparison of pre- and postoperative colonic biopsies, was possible in 22 patients. Although none of the appendices showed transmural inflammation, in most appendices evidence of active mucosal inflammation was found. There were 7 patients without any sign of appendiceal inflammation, or with only some characteristics of a previous inflammatory infiltrate (Geboes grade 0-1). There were four patients with extensive ulcerations or without any mucosal epithelium (grade 5). In 11 patients, there was active inflammation ranging from chronic inflammatory infiltrate with neutrophils and eosinophils (grade 2) to severe inflammation with crypt destruction and crypt abscesses (grade 4) **(table 2)**.

Immunohistochemistry of the resected appendices showed increased infiltration of CD4+ T-lymphocytes in all appendices graded 2-4 with the Geboes score, confirming the active inflammatory state. The results of the appendiceal IgA:IgG ratio were more heterogeneous, varying from 1:6 – 4:1 in these therapy-refractory patients. In general, the ratio in the appendix was lower when compared to the colon, and there was a trend towards a more normal (higher) ratio in the non-inflamed appendices.

Table 2. Pathologic evaluation

	N=30
Macroscopic inflammation of the appendix	3 (10)
Geboes score	23 (77)
No abnormality or some inflammatory infiltrate (grade 0-1)	6 (20)
Active inflammation (grade 2-4)	14 (47)
Crypt destruction, and erosions or ulcerations (grade 5)	4 (13)

Pathologic response

The postoperative colonic Geboes score was determined in endoscopic biopsies of 15 patients, and 7 colectomy resection specimens, and compared to preoperative scores. After a median 13.0 weeks (range 7-51), pathological response was seen in 11 patients (47.8%) with a median decrease of 2 points (range 1-3). There was no correlation between pathological and clinical response (p=1.0). Clinical response was only seen in 6 of the 11 patients with pathological response (p=0.7), with 3 patients undergoing colectomy for persistent symptoms having complete mucosal healing in the resection specimen. There were no clinical parameters predictive of pathological response, although a pathological response was more frequently seen in patients with limited disease (proctitis or left sided versus pancolitis: 7/10 versus 4/12, p=0.06), after shorter disease duration (7.9 years versus 10.8 years, p=0.3), and in younger patients (37.6 versus 43.1 years, p=0.2). In contrast, active appendiceal inflammation (Geboes grade 2-4) was highly predictive of pathological response when compared to no inflammation or extensive ulcerations (8/10 versus 3/12, p=0.02).

Immunohistochemistry showed that increased numbers of CD4+ T-lymphocytes and a decreased ratio of IgA:IgG in the appendix were predictive of pathological response, which was immunohistochemically characterized by an increased IgA:IgG ratio in colonic biopsies or resection specimens.

DISCUSSION

This study demonstrates that the appendix does play a role in UC, and that an appendectomy could be beneficial in therapy refractory UC patients. After 3 months, 57% of patients had a clinical response, which decreased to 40% after one year, with a substantial proportion of patients demonstrating complete endoscopic remission. Considering the fact that this patient group was referred for proctocolectomy, failing all regular medical treatments, these results seems promising. Although clinical response was the primary endpoint in this study, the pathological response was of particular interest. This study aimed to analyse which appendiceal characteristics and histological finding influenced the colonic response. Improvement in the colonic pathological Geboes score was seen in 50% of patients. Pathologic response was significantly related to active inflammation in the appendix, characterised by an increase in CD4+ T-lymphocytes and a decreased IgA:IgG ratio in the appendiceal resection specimens.

Unfortunately, the endoscopic finding of a cecal patch at the appendix orifice was not consistently scored preoperatively. This finding has been described in UC, predominantly in patients with distal colitis and has been considered to be a distinct ‘‘skip lesion’’. A recent review by Park et al. summarized all studies published until May 2012 regarding the role

of a cecal patch in UC. Thirteen studies evaluated endoscopic reports and demonstrated a prevalence varying between 10 to 75%.[11] The majority of studies demonstrate a similar disease course in patients with and without a cecal patch. Furthermore, the activity in the cecal patch seems to correlate with the distal colon. However, published data regarding the clinical significance remains controversial. Therefore, no particular therapeutic or monitoring recommendations could be made for patients with a cecal patch.

This study shows that pathological response was more frequently seen in patients with distal colitis when compared to patients with extensive colitis. This is in line with the results from the only other prospective series analysing the effect of appendectomy for severe proctitis.[6] Furthermore, pathologic response was more frequently seen in patients with shorter disease duration. This finding indicates that patients with a longer disease course may have more progressed disease that could become therapy refractory. This is in line with both murine as large epidemiological studies that demonstrated a beneficial effect of an appendectomy at a younger age.[8,12]

The current study showed a pronounced discrepancy between how patients feel and what is seen during colonoscopy and pathology. It is not uncommon to find that clinical symptoms and endoscopic findings do not correlate. Two prospective studies of 101 UC patients in remission and 54 UC patients with varying disease severity, demonstrated that serological markers (CRP and ESR) correlated well with the disease activity, while conflicting results were shown when comparing with endoscopic findings.[13,14] In the current study, it resulted in 3 colectomies, which showed full mucosal healing after pathological assessment. These patients insisted upon a colectomy after years of being unwell and not experiencing a clinical benefit from appendectomy in a short amount of time. Generally, a 3-month period is maintained to observe any effect of an appendectomy. However, 3 patients showed a late response after 12 months.

One of the theories linking the appendix to UC is the fact that the appendix is a major site for generation of IgA-secreting cells that migrate to the large intestine. In a mouse-model it was demonstrated that appendectomized mice show delayed and decreased accumulation of IgA^+ cells in the large intestine, resulting in an altered colonic faecal microbiota composition. It was suggested that this mechanism could be responsible for the beneficial effect of an appendectomy. However, this study shows that this finding cannot be extrapolated to the human situation, as the results were completely the opposite. After an appendectomy, there was an increase in the amount of sIgA, with an increasing ratio of IgA:IgG. As IgA is the most abundant class of antibodies found in the intestinal lumen of humans, this immune response has long been recognized as a first line of defence in protecting the intestinal epithelium from enteric pathogens and toxins. Restoring the normal ratio would therefore be beneficial, even though this may have been a readout of disease activity rather than a direct effect of the appendectomy. Considering

that the appendix may be the main source of SIgA production, we can hypothesize that an appendectomy may stimulate other gut-associated lymphoid tissues (GALTs) to induce plasma cell production for sIgA, therefore causing an increased IgA:IgG ratio in the colon.

Recently two reports have emerged that suggest that an appendectomy does not prevent colectomy.[15,16] In addition, an appendectomy could even be associated to an increased risk of high-grade dysplasia and cancer in the colon. As these are all retrospective series, the data are difficult to interpret and there are to many confounding variables to draw firm conclusions. The indication for appendectomy in these patients is not described. It could be speculated that it was offered to patients who failed all medical treatment, or patients with abdominal complaints mimicking appendicitis (representing perhaps a more severely affected group of UC patients). When looking at the timing of colectomy it is striking that the appendectomy group had a much longer duration of UC (151 versus 41 months), which could lead to speculation of a beneficiary effect of appendectomy, leading to a postponed colectomy comparable to the results of this study. This could also explain the higher incidence of cancer found in the colectomy specimens after appendectomy. The cancer risk is predominantly increased in pancolitis patients after longer follow up. Therefore, we do not think that this finding should lead to the conclusion that an appendectomy cannot be recommended for UC. But obviously, one should be aware that this patient group with therapy-refractory disease is at risk of developing a malignancy, and careful follow up and surveillance is mandatory.

The strength of the study is the prospective consecutive nature of the series, with the possibility of comparing pre- and postoperative biopsies. The weakness is the small number of patients, and the short follow up. Studies analysing the effect of a new treatment strategy for UC, often suffer from placebo effects up to 30%.[17,18] An invasive intervention like appendectomy in this patient group will definitely have some placebo effect, but all clinical results were correlated to pathological responses. Long-term FU will have to be awaited to analyse whether there is a sustained benefit in these patients and whether an appendectomy can be implemented as safe therapeutic option or as a bridge to proctocolectomy.

Appendectomy was effective in at least 30% of therapy refractory UC patients, with a substantial proportion of patients demonstrating complete endoscopic remission after 1 year. Considering the fact that these patients otherwise had to sacrifice their colon, this can be considered as a tremendous gain. Pathological response was related to active inflammation in the appendix, while there appears to be a discrepancy between clinical symptoms and what is seen during colonoscopy and pathology. If the inflammatory state of the appendix could be determined predicting pathological response to appendectomy, patients can be identified benefitting from resection. These early results suggest that UC patients with mucosal appendicitis may benefit from appendectomy. An appendectomy is

a relatively simple procedure with low morbidity that can be performed in day care setting. However, long-term follow up is warranted to exclude a possible placebo effect.

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CHAPTER 4

Lymphocytes populations in appendiceal lavage fluid predictive of IBD-related inflammation

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Submitted

ABSTRACT

Background: Appendectomy is currently studied as a therapeutic strategy for ulcerative colitis (UC). Mucosal inflammation with increased cytokine production within the appendix has been suggested to play a causative role in the development of relapses, which could be prevented by appendectomy. Measurement of T-cell infiltration in the appendix could be useful for clinical decision making. The objective of this study was to evaluate if appendiceal lymphocyte infiltration can be determined by analysis of lavage fluid and whether discrimination was possible between both types of IBD, acute appendicitis, and controls.

Methods: We studied the appendix of 41 patients, which was surgically removed during laparoscopy. Fifteen patients had UC (5 in remission), 9 Crohn's disease (CD), 7 acute appendicitis (AA), and 10 were non-inflammatory controls undergoing resection for cancer in the ascending colon. The appendix and cecal base were isolated from the surgical resection specimen and flushed with 2cc of phosphate buffered saline. Presence of CD4+ and CD8+ lymphocytes in the lavage fluid was determined by FACS analysis and the CD4+/CD8+ ratio was calculated. Mucosal and transmural inflammation of the appendix wall was determined by routine H&E histology and graded according to the validated Geboes score (ranging from 0 = no inflammation to 5 = erosion or ulceration). The appendices were also immunohistochemically stained for CD4 and CD8, after which mucosal lymphocyte influx was graded from 1 (low cell count) to 4 (high cell count). Results were correlated to clinical disease activity.

Results: The appendices of UC patients showed mucosal inflammation in 80% (12/15, Geboes score ≥2) despite a macroscopically normal appearance. In all CD and AA patients, inflammation was transmural rather than mucosal. No inflammatory features were found in controls. Immunohistochemistry demonstrated increased mucosal CD4+ lymphocytes (grade 2 – 4) in patients with active inflammation, with no significant difference among active UC (9/10), CD (8/9) and AA (6/7) patients. Since increased mucosal CD4+ lymphocytes were only found in 2/5 UC patients in remission and 3/10 controls, this parameter correlated to clinical disease activity ($r = 0.70$, $p<0.001$). A high proportion of CD4+ lymphocytes in the lavage fluid was predictive for mucosal inflammation (Geboes score >2) ($p<0.001$). IBD (UC and CD) patients had a significantly increased CD4+/CD8+ ratio in the lavage fluid compared to non-IBD patients (AA and non-inflammatory controls) (6.4 versus 4.3, $p=0.007$). This increased ratio was found in both active UC patients and patients in remission and therefore could discriminate IBD from non-IBD patients.

Conclusion: Despite a macroscopically normal appearance, appendices of most UC patients show histological characteristics of mucosal inflammation, with increased mucosal CD4+ lymphocytes. An increased CD4 proportion in appendiceal lavage fluid, was predictive of a high appendiceal Geboes score in UC patients, and correlated with clinical and immunohistochemical findings in UC, CD and AA patients. In addition, IBD patients show a distinct immunological profile with increased CD4+/CD8+ ratio. If an appendiceal phenotype could be determined predicting clinical response to appendectomy, lavage fluid could be used to identify patients benefitting from resection.

INTRODUCTION

Until recently the appendix was mostly seen as a rudimentary part of the human intestine, but nowadays it has been demonstrated to have a distinct immunological function. Reports are emerging linking this vermiform organ to the development of ulcerative colitis (UC) and a systematic review suggests that an appendectomy could modulate the disease course.[1] In addition, various animal studies have shown that the removal of the appendix prevents the development of experimental colitis, which further supports this hypothesis.[2,3]

Although the pathogenesis of UC is not fully understood, evidence suggests that the activated immune system is mostly mediated by lymphocytes with a Th2 like phenotype.[4] Extensive infiltration of subgroups of CD4+ T cells and elevated cytokine proportions have been observed in the inflamed mucosa of UC patients.[5,6] Although characteristic transmural histological changes are hardly ever seen in appendectomy specimens of UC patients, a quantitative and qualitative change of the lymphocyte phenotype has been described. A previous study that characterized the histological and immunological characteristics of the appendix in UC patients demonstrated that the various degrees of inflammation were similar to those found in the colon and rectum.[7]

In contrast, for patients with Crohn's disease (CD) higher incidence rates after appendectomy have been described.[8] However, these data are difficult to interpret since the appendix is frequently involved as part of terminal ileitis, which could result in overestimated incidence rates.[9–11] Most specimens show macroscopically and microscopically affected appendices with transmural inflammation, which is comparable to the affected terminal ileum.

Acute appendicitis (AA) represents a different form of transmural inflammation. This non-autoimmune coordinated inflammation has been linked to bacterial invasion, diet, familial aggregation and an obstructing appendiceal faecolith, which possibly play a role in the aetiology of the disease.[12–14]

To gain insight in the distinct role of the appendix in the development of UC, it would be interesting to compare immunological changes between inflammatory and non-inflammatory specimens. The scarce literature on human UC appendices only discusses inflammatory characteristics in resection material. However, If T-cell infiltration and characterization could be clinically determined in the appendix, it might be possible to utilize this as a measurement for the inflammatory process, and guide clinical decision making. Ideally, the immunological phenotype could be used to predict clinical response to appendectomy. During colonoscopy, the orifice of the appendix can be identified which creates the possibility to perform an appendiceal lavage with additional FACs analysis of lymphocyte populations.

The objective of this study was to evaluate if appendiceal lymphocyte infiltration can be determined by analysis of lavage fluid and whether discrimination was possible between UC (remission and active), CD, acute appendicitis, and non-inflammatory controls.

MATERIALS AND METHODS

Patient selection

This prospective cohort study was performed in a tertiary IBD centre between August 2011 and December 2015. UC patients over 18 years of age with therapy refractory UC scheduled for elective colectomy and UC patients in remission participating in the ACCURE trial (a randomized controlled trial analysing the effect of an appendectomy in maintaining remission in UC patients, trial register; NTR2883) were included.[15] The control groups consisted of patients with CD undergoing ileocolic resection, AA patients undergoing laparoscopic appendectomy, and patients undergoing (partial) colectomy for colonic carcinoma or familial adenomatous polyposis (FAP). Demographic data such as gender, age, medication use and details concerning the extent and duration of the disease was registered. Each patient signed an informed consent form approved by the institutional review board.

Clinical disease activity

In UC patients, disease activity was determined by the full Mayo score measured within 3 months before surgery.[16] Active disease was defined as a score of > 5 (with at least an endoscopic subscore of ≥ 2). In CD patients, active disease was defined as inflammation shown on recent CT and/or MRI imaging and endoscopy. Patients with a stenosis, without inflammation were defined as inactive. In AA patients, only macroscopically affected appendices found during surgery were resected. All control patients were defined as inactive disease.

Surgical procedure

Surgical procedures were performed by or under supervision of a dedicated gastrointestinal surgeon. Care was taken not to touch the appendix during dissection. After resection, the specimen was extracted from the abdominal cavity and the appendix was removed from the resection specimen under sterile conditions. The mesentery of the appendix was removed and the appendicular tissue was cleaned of peri-appendicular fat. The distal tip of the appendix was cut off to enable flushing the appendix with fluid. The appendix was inserted in a transparent tube to provide circular pressure during flushing. Subsequently, the appendix was flushed with 2cc of phosphate buffered saline (PBS). In case of faecal contamination of the fluid, a second flush with 2cc of PBS was performed. The lavage fluid was collected in a container with a protein medium and analysed in the clinical chemical

laboratory of the AMC. Subsequently, the appendix was transported to the department of pathology for histological evaluation and immunohistochemical staining.

Histology

Formalin-fixed and paraffin-embedded appendiceal specimens were cut and H&E-stained in preparation for histological evaluation according to standard operating procedures. Tissue was evaluated by a dedicated gastrointestinal pathologist assessing architectural and inflammatory features. Inflammation was graded according to the validated Geboes scoring system, which subdivides 6 grades based on structural (architectural) change: no abnormality, chronic inflammatory infiltrate, lamina propia neutrophils and eosinophils, neutrophils in epithelium, crypt destruction, and erosions or ulcerations.[17] Scores can range from 0 to 5, with higher scores indicating more severe histological inflammation ulceration. Active inflammation was defined as grade 2 or higher. Although the Geboes score has only been validated for UC, this scoring system was also used for CD and AA to grade the severity of inflammation to ensure comparability. No other validated grading scales are available for these conditions.

Immunohistochemistry

Paraffin embedded slides were stained with CD4 and CD8 antibodies (Biolegend, San Diego, CA) for immunohistochemical analysis. Results were assessed by three reviewers (SS, HH and CB) independently, blinded for the patients' clinical records and disease diagnosis, and scored according to the number of positive cells per high power field. A representative mucosal area was chosen which was not directly covering a lymphoid follicle in the submucosa or lamina propria of the appendiceal wall. Scores were adapted from Stumpf et al. (grade 1 representing no staining and grade 4 extensive lymphocyte infiltration).[18] Increased infiltration was defined as a score of > 1. The cases in which no consensus was reached regarding the grading, a fourth member was asked to evaluate the samples (MV) (**Figure 1**).

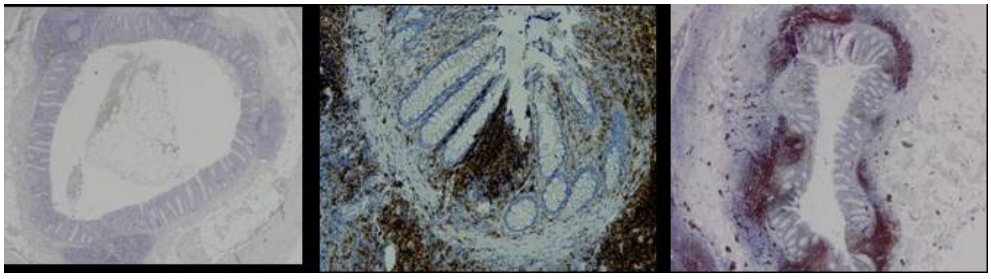

Figure 1. Immunohistochemical analysis, CD4+ T cell are stained in this image (brown coloured cells). In image A, a normal appendix is shown. The appendix of patients with appendicitis showed extensive influx (grade 3 or 4) of CD4+ cells (B). Also in the appendix of UC patients, extensive CD4+ influx can be seen (C).

Appendiceal lavage fluid analysis

Mononuclear cells in the lavage fluid were stained with fluorescein is thiocyanate (FITC) and phycoerythrin-conjugated (PE) monoclonal antibodies to CD45+, CD3+, CD4+ and CD8+ cells (Biolegend, San Diego, CA). First the phenotyped cells were analysed by flow cytometry (FACS analysis). Cell suspensions were visualised in the forward scatter/side scatter profile, subsequently lymphocytes (CD3+ cells) were gated. The proportion of CD4+ and CD8+ T-cells were determined and the relative ratio of CD4+ and CD8+ T-cells was calculated (**Figure 2**).

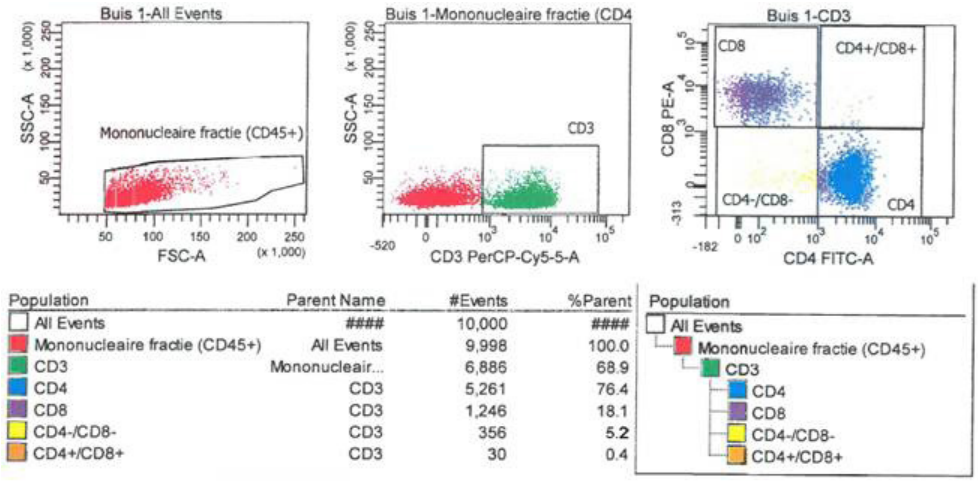

Figure 2. Image of a colour flow cytometry of appendix lavage fluid. Cell suspensions were visualised in the forward scatter/side scatter profile, subsequently lymphocytes were gated. The proportion of CD4+ and CD8+ T cells and the CD4/CD8 ratio in the total lymphocyte populations were calculated.

Statistical analysis

Categorical data are presented as frequencies and percentages. Continuous data are presented as mean and standard deviation (SD) or as median and interquartile range (IQR) according to the distribution. Several comparisons were made; UC patients were compared to all other patients groups, active cases (active UC, CD and AA) were compared to non-active cases (UC in remission and non-inflammatory controls) and IBD cased (UC and CD) were compared to non-IBD cases (AA and non-inflammatory controls). Independent t-test was used to compare means. Mann-Whitney-U test was used for continuous, not normally distributed data. The Kruskal Wallis test was used when more than 2 groups were compared. To compare dichotomous data the $\chi 2$-test or Fisher's exact test were used. The optimal cut-off value for increased lavage CD4+ T cell proportion discriminating between active and inactive disease was determined using receiver operating characteristic (ROC) analysis and determining the highest Youden index. The Youden index is calculated by the following formula: (sensitivity + specificity) – 1. Pearson correlations were used to examine

relationships between disease activity, immunohistochemistry and lavage. The following crude estimates are generally used for interpreting strengths of correlations: if r = 0.70 or higher, there is a very strong relationship; if r = 0.40 to 0.6, there is a strong relationship, if r = 0.30 to 0.39, there is a moderate relationship; if r = 0.20 to 0.29, there is a weak relationship and if r = 0.01 to 0.19, there is no or negligible relationship. All tests were analysed two-sided and a P-value of <0.05 was deemed significant. Statistical analysis was done with IBM SPSS Statistics for Windows®, Version 22.0 (IBM Corp., Armonk, NY, United States).

RESULTS

Demographics

A total of 41 patients were included; 15 UC patients, 9 CD patients, 7 AA patients and 10 non-inflammatory controls (colonic carcinoma or FAP). Five UC patients were in remission with a median mayo score of 3 (IQR, 3 -4) and 10 UC patient had clinically active disease with a median mayo score of 11 (IQR, 10 – 11). All 9 CD patients had active disease of which 4 also showed stenosis. The characteristics of the patient groups are summarized in **table 1**.

Table 1. Baseline patient characteristics

	IBD			Non-IBD	
	UC		CD	AA	Controls
	Remission	Active			
Total *n (%)*	5	10	9	7	10
Male	2 (40)	4 (40)	2 (22)	4 (57)	5 (50)
Age at surgery*	43 [26-63]	43 [40-54]	25 [21-31]	43 [38-52]	61 [56-70]
Extent of UC					
- Proctitis	2 (40)	2 (20)			
- Left sided	1 (20)	3 (30)			
- Pancolitis	2 (40)	5 (50)			
Disease activity					
- Mayo score*	3 [3-4]	11 [10-11]			
- Stenosis			4 (44)†		
Disease duration *(months)**		66 [25-219]	19 [13-93]	NA	1 [0-1]
Medication					
- None	4 (80)	0 (0)	1 (11)		
- Steroids	1 (20)	6 (60)	2 (22)		
- Immunomodulators	0 (0)	1 (10)	2 (22)		
- Anti-TNF	0 (0)	3 (30)	3 (33)		
- Combination	0 (0)	0 (0)	1 (11)		

*Median and IQR

Histology

The appendices of all UC patients appeared macroscopically normal, but showed mucosal based inflammation with Geboes score ≥ 2 in 12/15 patients. In contrast, all CD and AA patients demonstrated macroscopically abnormal appendices with an increased diameter, thickened meso-appendix, and a fibrino-purulent exudate covering the serosa. Histology confirmed the transmural inflammation with oedema and lymphocyte influx present in the macroscopically affected appendices. The appendices of all non-inflammatory controls were both macroscopically and histologically normal. The outcome measures are shown in **table 2**.

Table 2. Outcome measures

	IBD			**Non-IBD**	
	UC		**CD**	**AA**	**Controls**
	Remission	**Active**			
Total *n (%)*	5	10	9	7	10
Histology *n (%)*					
Macroscopically inflamed	0 (0)	0 (0)	9 (100)	7 (100)	0 (0)
Microscopically inflamed	4 (80)	8 (80)	9 (100)	7 (100)	0 (0)
Immunohistochemistry *n (%)*					
CD4+ cell count					
- Grade 1	3 (60)	1 (10)	1 (11.1)	1 (14.3)	7 (70)
- Grade 2	0 (0)	6 (60)	2 (22.2)	4 (57.1)	3 (30)
- Grade 3	2 (40)	3 (30)	3 (33.3)	1 (14.3)	0 (0)
- Grade 4	0 (0)	0 (0)	3 (33.3)	1 (14.3)	0 (0)
CD8+ cell count					
- Grade 1	1 (20)	1 (10)	2 (22.2)	1 (14.3)	1 (10)
- Grade 2	4 (80)	7 (70)	7 (77.8)	5 (71.4)	8 (80)
- Grade 3	0 (0)	2 (20)	0 (0)	1 (14.3)	1 (10)
- Grade 4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lavage* *mean [SD]*					
T cells					
CD4+ proportion	69 [12]	73 [15]	77 [11]	69 [12]	65 [13]
CD8+ proportion	11 [5]	14 [5]	13 [4]	18 [6]	18 [7]
CD4+/CD8+ ratio	7 [2]	6 [2]	7 [3]	4 [2]	4 [2]

*Missing: 4 UC, 2 CD and 2 control

Immunohistochemistry

Representative examples of immunohistochemical results are presented in Figure 2 (grade 1 – 4). There was a 91.7% consensus on discrimination between grade 1 (no increased influx) and grades 2 – 4 (increased influx). Immunohistochemistry demonstrated that the increased number of lymphocytes seen at histology were predominantly CD4+ T cells. The appendices of patients with active disease (active UC, CD and AA) showed a significantly increased influx of mucosal CD4+ T cells (grade 2 – 4) with no significant discrepancy between active UC (90%), CD (89%), and AA patients (86%) (Figure 3). Since increased mucosal CD4+ T cells were only found in 2/5 UC patients in remission and 2/8 non-inflammatory controls, this was highly predictive of clinical disease activity ($r = 0.70$, $p<0.001$). On immunohistochemistry, no significant differences could be found in the CD8+ lymphocytes between all patient groups, although a relative increase was suggested in AA (**Table 2**). Due to the categorical histopathological scoring, no meaningful CD4/CD8 ratio could be calculated.

Appendiceal lavage

In 7 patients (3 UC, 2 CD and 2 control) analysis of the lavage fluid could not be performed due to luminal obliteration or excessive faecal contamination and therefore technical flushing problems. Comparable to immunohistochemical results, the median proportion of CD4+ lymphocytes in the clinically active cases (active UC, CD, AA) were significantly higher than non-active cases (UC remission and non-inflammatory controls), with no significant difference between the mean proportion of CD4+ lymphocytes between IBD and non-IBD patients ($p> 0.05$). The optimal cut-off value to discriminate between active and inactive disease was > 56% (sensitivity 0.94 and specificity 0.37) for CD4+ lymphocytes per lavage. CD4 proportion in the lavage correlated significantly with clinical disease activity ($r= 0.42$, $p = 0.018$), with the Geboes score ($r=0.65$, $p=0.012$), and with CD4+ lymphocyte grading on immunohistochemistry ($r = 0.43$, $p = 0.033$). A high CD4 proportion in lavage was predictive for a high Geboes score (OR 40; $p=0.002$). In the lavage fluid, IBD patients (UC and CD) had relatively low CD8+ proportions compared to non-IBD patients (AA and non-inflammatory controls) (17.8 versus 13.0, $p=0.022$) resulting in a significantly increased CD4+/CD8+ ratio for IBD patients (6.4 versus 4.3, $p=0.007$) (**Figure 3**). This increased ratio was found in both active and non-active UC patients and therefore could discriminate IBD from non-IBD patients.

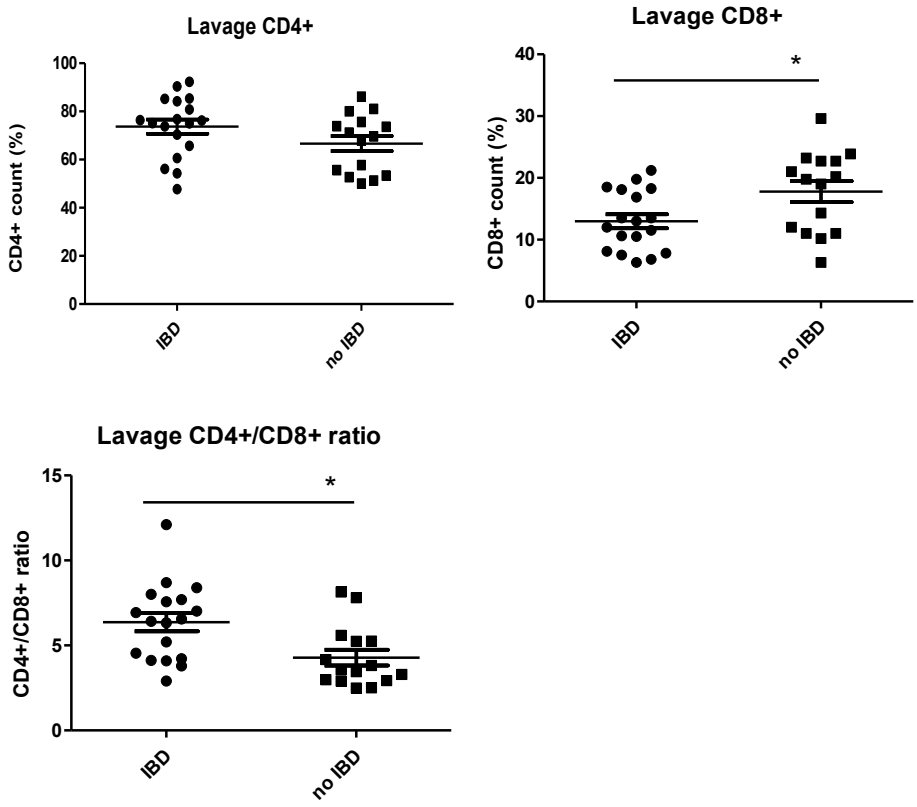

Figure 3. Proportions of CD4+ lymphocytes, CD8+ lymphocytes and CD4/CD8 ratio in the lavage fluid. Comparisons are made between IBD and non-IBD patients. The asterisk indicates a significant difference.

DISCUSSION

The link between the appendix and UC has been demonstrated in various epidemiological studies, but the etiological connection remains unknown. The aim of this study was to characterize the T-cell phenotype in appendiceal tissue, correlate findings to clinical disease activity, and analyse if appendiceal lavage fluid was predictive of the immunological profile compared to the gold standard histology. As hypothesized, we found that despite a macroscopically normal appendix, most UC patients have mucosal inflammation and increased numbers of mucosal CD4+ lymphocytes in immunohistochemical staining of resected appendices. An increased CD4 proportion in appendiceal lavage fluid, was predictive of a high appendiceal Geboes score in UC patients, and correlated with immunohistochemical findings in UC, CD and AA patients. In contrast, increased levels of

CD8+ lymphocytes were only seen in AA, resulting in an increased CD4+/CD8+ ratio of the lavage fluid of IBD patients. This skewing of CD4+/CD8+ ratio was seen in patients with both active and remissive UC and in patients with CD.

Several theories have been proposed that explain the immunomodulating role of the appendix in UC.[1] One of the theories that has been suggested is that inflammation in the appendix may trigger inflammation in colonic mucosa by releasing inflammatory mediators into the appendiceal lumen.[19] The appendix is known to be part of the gut-associated lymphoid tissue and is predominantly populated by naive T and B cells.[20,21] Here, T-lymphocytes are likely to be activated by various luminal antigens and may regulate the immunoglobulin-A (IgA)-producing B cells that home to the colon.[22] Although it is not clear how a dysbalance of IgA may predispose to UC, it is interesting to hypothesise that removal of the appendix could mitigate this response. A previous study supports this theory by demonstrating an increased proportion of immature plasma cells in the appendix of UC patients, irrespective of disease activity, suggesting a possible primary role of humoral immune responses in the pathogenesis of UC. In addition, they showed increased numbers of proliferative lymphocytes (Ki-67+CD3+ cells) in the appendix of UC patients compared with CD, AA and non-inflammatory control patients, which also indicated involvement of T cell lineage.[23] Another interesting theory is that the appendix may act as a 'safe house' for commensal bacteria that could repopulate the gut under certain conditions. This phenomenon has been observed in patients with recurrent Clostridium difficile infections. In UC patients, appendiceal microbiota may cause inflammation due to the deranged mucosal immune system and impaired barrier function.[24] In this sense, the appendix could presents as a priming site for UC.

A study by Matsushita et al. also demonstrated an increased CD4+/CD8+ ratio in appendix biopsies of UC patients with active left sided colitis, when compared to non-inflammatory controls.[25] Interestingly, as the CD4+/CD8+ ratio in the appendix increased, the ratio in the rectum tended to increase as well. These results have led to the suggestion that the CD4+/CD8+ ratio in the mucosa of the appendix represents the severity of inflammation in the colonic mucosa. Although we could not demonstrate a higher CD4+/CD8+ ratio in active disease, the finding of an elevated CD4+/CD8+ ratio in UC patients in remission is intriguing. If indeed this appendiceal immunological dysbalance would contribute to the initiation of UC relapse, this could explain why reduced relapsing rates have been described after appendectomy.[26,27]

Unfortunately, an appendectomy will not be effective in all UC patients. The best results so far have been described by Bolin et al, with 40% clinical remission after appendectomy.[28] Appendiceal resection also harbours the risk of surgical complications, and recently a retrospective study suggested that appendectomized UC patients might have an increased risk of colorectal cancer.[29] Although these results from a small single centre study might have suffered from inclusion bias and results could be influenced by confounders since

appendectomized patients had longer follow up with their colon in situ, it also demonstrates that it is of crucial importance to identify patients possibly benefitting from resection, without exposing all UC patients to the possible disadvantages from appendectomy. Therefore, demonstrating an aberrant immunological profile with increased proportion of CD4 lymphocytes in lavage fluid could guide clinical decision making.

The CD4+/CD8+ ratio has been used as a diagnostic tool in a variety of diseases, including pulmonary, HIV and autoimmune diseases.[30–33] In these diseases, the CD4+/CD8+ ratio is used as an indicator for immune dysfunction. An intriguing disease that shows analogy with autoimmune diseases is sarcoidosis, a systemic granulomatous disease of unknown aetiology that primarily affects the lung. The sarcoid reaction is characterized by accumulation of activated T cells and macrophages at sites of inflammation compared to other interstitial lung diseases such as hypersensitivity pneumonitis in which the lung infiltrates are characterized by cells bearing suppressor/cytotoxic phenotype.[34] A previous study investigated the clinical usefulness of bronchoalveolar lavage (BAL) cellular analysis with lymphocyte subsets and showed a significant increase in the percentage of CD4+ cells, a decrease in CD8+ cells, and an increase in the CD4+/CD8+ ratio in patients with sarcoidosis compared to other interstitial lung disease.[35] The elevated CD4+/CD8+ ratio in BAL fluid may confirm the diagnosis in patients presenting with a typical picture of sarcoidosis. These findings are in line with our data for IBD.

In this study, we have prospectively identified patients with different inflammatory and non-inflammatory diseases and evaluated their appendiceal tissue. The appendices were evaluated on all levels; macro- and microscopically and by lavage of the appendiceal lumen. Assessment of the histological and immunohistochemical samples was blinded for diagnosis to avoid review bias. The intra- and inter-observer measurement error variability was restricted by independently repeating the sample scoring three times and by using three observers.

Unfortunately, the small numbers in every group precluded correlation with varying inflammation grades of disease. Nevertheless, we are the first that have adopted the lavage method on appendices in order to differentiate between diseases of the gastrointestinal tract.

In conclusion we found that despite a macroscopically normal appearance, appendices of most UC patients show histological characteristics of mucosal inflammation, with increased mucosal CD4+ lymphocytes. An increased CD4 proportion in appendiceal lavage fluid, was predictive of a high appendiceal Geboes score in UC patients, and correlated with clinical and immunohistochemical findings in UC, CD and AA patients. In addition, IBD patients show a distinct immunological profile with increased CD4+/CD8+ ratio. If an appendiceal phenotype could be determined predicting clinical response to appendectomy, lavage fluid could be used to identify patients benefitting from resection.

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PART II

Disease behaviour, quality of life and the risk of colectomy

CHAPTER 5

Risk factors for proximal disease extension and colectomy in left-sided ulcerative colitis

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ABSTRACT

Objective: The primary objective of this study was to assess proximal disease extension of ulcerative colitis (UC) over time, with disease behaviour pattern and risk factors for proximal disease extension and colectomy as secondary aims.

Methods: All cumulative incident cases diagnosed with UC at the Academic Medical Center between January 1990 and December 2009 were studied. The cumulative risk of colectomy was calculated by Kaplan-Meier analysis. The cox proportional hazards regression was used to identify risk factors associated with proximal disease extension and colectomy.

Results: In total, 506 UC patients were included with a median age of 33 years (IQR 23 – 41) at diagnosis. Ninety-five (18.8%) patients underwent colectomy during follow up. Median follow up was 10 years (IQR 5 – 15). Initial disease extent was evaluable in 416 patients, of whom 142 (34.1%) had proctitis, 155 (37.3%) left sided colitis and 119 (28.6%) pancolitis. Proximal disease extension was observed in 120 (28.8%) patients during follow up (51 proctitis to left-sided colitis, 39 proctitis to extensive colitis and 30 left sided to extensive colitis). Disease behaviour was evaluable in 378 patients, of whom 244 (64.6%) had less than 1 relapse per year. Younger age at diagnosis (HR 0.98, 95%CI 0.96 – 0.99) and continuous active disease (HR 2.18, 95%CI 1.27 – 3.73) were independent risk factors for proximal disease extension. The cumulative risk of colectomy did not change over time between patients diagnosed before and after the year 2000 (p=0.341). Continuous active disease (HR 7.05, 95% CI 4.23 – 11.77), systemic steroids (HR 3.25, 95% CI 1.37 – 7.71) and cyclosporine treatment (HR 2.80, 95% CI 1.66 – 4.72) were independent risk factors for colectomy, whereas proctitis at diagnosis (HR 0.43, 95% CI 0.22 – 0.86) carried a lower risk.

Conclusion: In one third of UC patients, left-sided disease at diagnosis will extend proximally during 10 years of follow up. Proximal disease extension was not a risk factor for colectomy, but the risk of colectomy is rather determined by continuous disease activity, and use of systemic steroids and cyclosporine.

INTRODUCTION

Ulcerative colitis (UC) is diagnosed in approximately 9-20 patients per 100.000 inhabitants per year in Northern Europe and North America and poses a formidable burden on the lives of young adults.[1] The clinical presentation at onset of the disease and the subsequent disease course varies significantly among patients. In general, the disease course of UC is characterized by a relapsing and remitting behaviour, but up to 6% of the patients experience chronic continuous symptoms.[2] Furthermore, distal colitis may progress towards the proximal colon, whereas extensive UC may regress over time. This pattern is difficult to predict and may influence the prognosis and the necessity for colectomy.

The ECCO guidelines recommend the use of the Montreal classification for defining the distribution of disease to describe the maximal proximal disease extent of inflammation seen at colonoscopy.[3] Using this classification system, earlier series have shown that approximately 30-50% of UC patient have disease confined to the rectum, 20-30% have left sided colitis and 20-30% have disease that extends beyond the hepatic flexure (then named 'extensive colitis' or 'pancolitis').[4,5] More recent studies have shown a preponderance for left sided colitis and suggested that an initial presentation with extensive colitis could be a risk factor for colectomy.[6,7] Knowledge about the extent of the disease is essential to determine the optimal treatment strategy (e.g. appropriateness of topical treatment) and estimate the need for colectomy in the near future.

Over the last two decades, medical treatment has evolved from suppression of symptoms, mainly with sulfasalazine, 5-aminosalycates and glucocorticosteroids, to more immunomodulatory and targeted therapies such as anti-TNF and integrin antibodies.[8] Since most of the previously mentioned studies have been performed before the era of biologicals, it remains unknown to what extent the disease course and colectomy rates have changed in more recent years. Furthermore, studies that have investigated disease behaviour and extent over time are limited in number. It has been suggested that patients that experience chronic active symptoms or have disease that extends proximally over time may have an aggressive phenotype and an increased risk of colectomy. If the behaviour of UC and the risk for colectomy could be predicted, patients could be treated more appropriately earlier during their disease course.

Therefore, the objective of this study was to assess the progression of extent of disease and disease behaviour patterns and to identify prognostic risk factors for proximal disease extension and colectomy.

METHODS

Patient population

In this retrospective study, we included all UC patients treated at the Academic Medical Center (AMC) in Amsterdam, The Netherlands, between January 1990 and December 2009. Patients in the AMC are registered at initial presentation by diagnosis code. Patients diagnosed with unclassified inflammatory bowel disease (IBD-U), Crohn's disease (CD), microscopic colitis or infectious proctitis were excluded. Patients in whom the date of diagnosis was unknown were also excluded. Medical records were reviewed to obtain additional data. The demographic variables that were extracted included gender, age, smoking habits (current, stopped, never), body mass index (BMI), comorbidity grouped by organ system and extra intestinal manifestations. Disease specific data on disease localization, behaviour, and medical and surgical treatment were also registered.

Disease characteristics

All colonoscopy reports were reviewed to determine the UC disease extent and activity at diagnosis and during follow up. Disease extent was categorized according to the Montreal Classification: E1 (proctitis); distal to the sigmoid, E2 (left sided); distal to the splenic flexure, and E3 (extensive); proximal to the splenic flexure.[9] A colonoscopy was defined as inconclusive if the upper limit of inflammation was not reached. An exception was made when the scope reached the hepatic flexure and the upper limit was not seen; these cases were scored as extensive colitis.

Proximal disease extension was defined as inflammation extending proximally from proctitis to left sided colitis, from proctitis to extensive colitis or from left sided colitis to extensive colitis. A relapse was defined as the occurrence of blood and mucus in the stool. Disease behaviour has been previously classified in 4 different patterns in the IBSEN study, in which the patients were asked to choose the curve that best described the course of their disease during this period.[10] In the current study, we determined the disease behaviour based on patients charts and categorized it according to the periods of exacerbation and remission as shown in **figure 1**; A) remission after diagnosis, B) less than 1 relapse per year, C) more than 1 relapse per year and D) continuous activity.

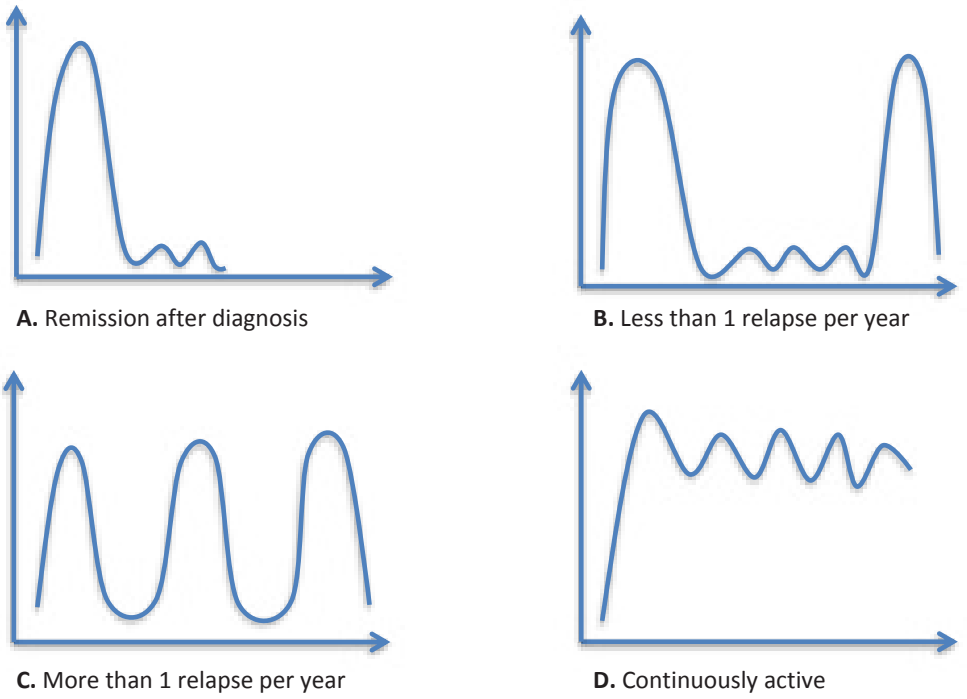


Figure 1. Disease behavior in UC patients: A) remission after diagnosis, B) less than 1 relapse per year, C) more than 1 relapse per year and D) continuous activity

Medical treatment

We registered the use of 5-aminosalycilic acid (5-ASA), glucocorticosteroids (ever received topical and systemic treatment), immunomodulators (azathioprine, 6-Tioguanine, 6-Mercaptopurine and methotrexate), cyclosporine and various biologicals.

Outcome measures

Our primary outcome measure was the incidence of proximal disease extension. The secondary outcome measures included disease behaviour, risk factors for proximal disease extension and risk factors for colectomy.

Statistical Analysis

Data were presented as counts and percentages or medians and interquartile ranges (IQR). For the purpose of the Kaplan-Meier and the Cox proportional hazard analyses, a 'time to extension' and 'time to colectomy' variable was created. Patients were censored if at the end of the follow-up period no proximal extension or colectomy had occurred, if they had died or were lost to follow up. Kaplan–Meier curves of patients that had been diagnosed

with UC before and after the year 2000 were compared by log rank test to evaluate whether the rates have changed over time. The year 2000 was chosen since this was half way of the time span of the current cohort, which coincided with the advent of biological treatment era. The cox proportional hazards regression was used to identify risk factors associated with proximal disease extension. Hereby, patients with extensive disease at diagnosis were excluded from this analysis. All medicinal variables were scored as 'yes' when patients were treated before proximal disease extension had occurred. The cox proportional hazards regression was also used to identify risk factors associated with colectomy. In this analysis, all patients that were indicated for colectomy due to dysplasia or cancer were excluded from the analysis. In univariable analysis, variables with a p-value of less than 0.2 were considered for multivariable analysis. Independent risk factors were identified by forward selection and considered to be significant with a two-sided p value of <0.05. Analysis was performed using IBM SPSS for Windows version 22 (SPSS Inc., Chicago, IL, USA).

RESULTS

Study population

In total, 888 patients were identified through the hospital registry and 506 UC patients (49.8% male) were included in the study with a median age of 33 years (IQR 23 – 41) at diagnosis. Fourteen patients with IBD-U, 80 CD patients, 4 patients with microscopic colitis, 1 with infectious proctitis and 283 patients in whom the date of diagnosis was unknown were excluded (**Figure 2**). The median duration of follow up was 10 years (IQR 5 – 15). Eight (1.6%) patients were diagnosed with gastrointestinal cancer during follow up of which 3 were localized in the sigmoid. Eight (1.6%) patients died during follow up, all due to other causes than UC. The demographic characteristics of the study cohort are presented in **table 1**.

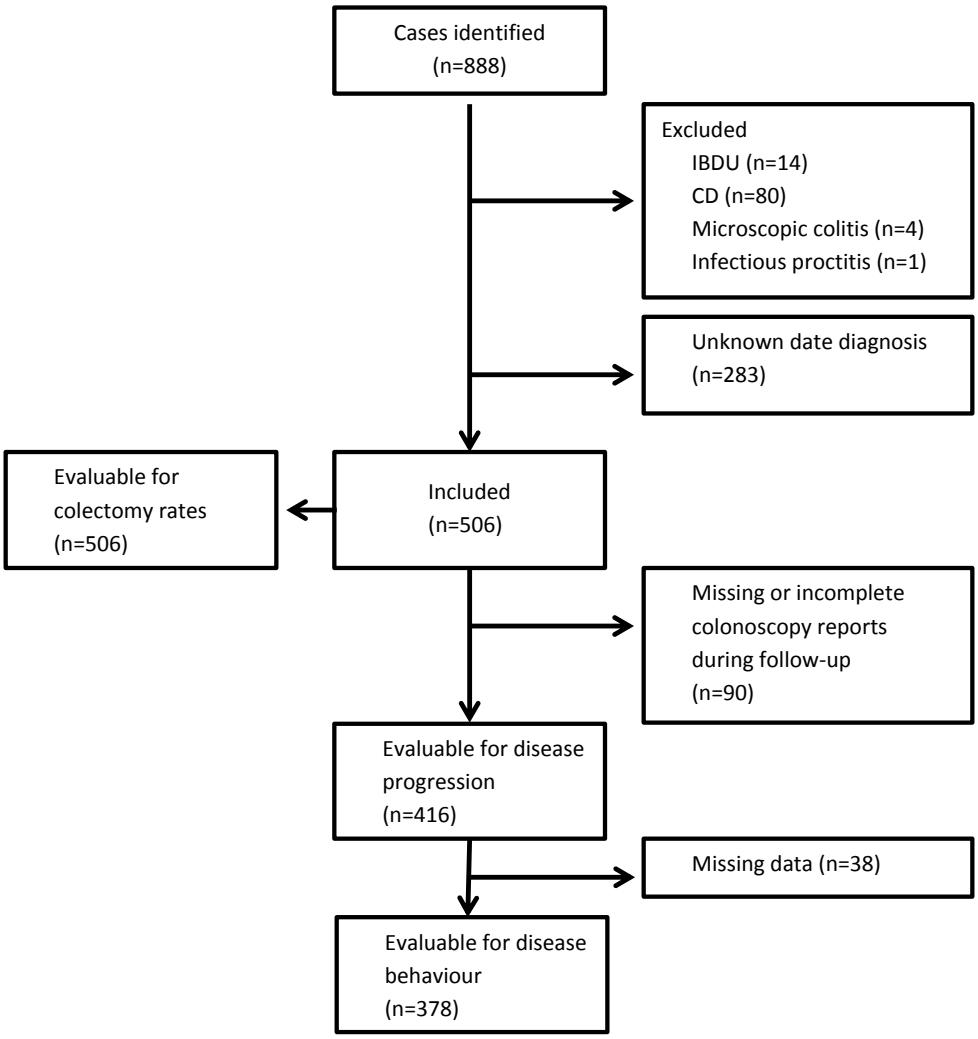


Figure 2. Flow diagram of patients included in the study

Table 1. Patient characteristics

	Total cohort	Colectomy	No colectomy
Total *n (%)*	506 (100)	95 (18.8)	411 (81.2)
Male	252 (49.8)	51 (53.7)	201 (48.9)
Age at diagnosis*	33 [23 – 41]	29 [21 – 39]	32 [23 – 43]
Age at colectomy*	36 [29 – 43]		
Smoking			
Never	248 (58.8)	46 (64.8)	202 (57.5)
Stopped	82 (19.4)	10 (14.1)	72 (20.5)
Current	92 (21.8)	15 (21.1)	77 (21.9)
EIM	69 (15.1)	11 (14.3)	58 (15.2)
PSC	17 (3.7)	6 (7.7)	11 (2.9)
Diabetes	10 (2.0)	0 (0.0)	10 (2.6)
Medication			
Systemic steroids	294 (62.2)	71 (86.6)	223 (57.0)
Topical steroids	237 (51.5)	42 (62.7)	195 (49.6)
5-ASA	473 (96.9)	74 (92.5)	399 (97.8)
Immunomodulators	265 (54.6)	63 (75.9)	202 (50.2)
Cyclosporine	60 (12.5)	27 (33.8)	33 (8.3)
Biologicals	104 (21.6)	33 (41.3)	71 (17.7)
Dysplasia	4 (0.8)	4 (4.2)	0 (0.0)
Cancer	8 (1.6)	6 (6.1)	2 (0.5)
Mortality	8 (1.6)	2 (2.1)	6 (1.5)
Follow up (years)*	10 [5 – 15]	4 [2 – 9†]	11 [7 – 16]

* Median and IQR
† FU stopped at the time of colectomy
Missing values: smoking (n=84); EIM (n=48); PSC (n=47); appendectomy (n=65); topical steroids (n=46); systemic steroids (n=33); 5-ASA (n=18); Immunomodulators (n=21); cyclosporine (n=26); Anti-TNF (n=24)

Proximal extension

Extent of disease could not be defined in 90 of 506 (17.7%) patients, because colonoscopy reports were missing or contained insufficient details. This resulted in 416 evaluable patients for disease progression. At diagnosis, proctitis was present in 142 of these patients (34.1%). During follow up, 51 (35.9%) progressed to left-sided colitis and 39 (27.5%) to extensive colitis. Initial left sided UC was diagnosed in 155 (37.3%) patients. Thirty of these patients (19.4%) progressed to extensive colitis. Extensive disease at primary diagnosis was found in 119 (28.6%) patients (**Figure 3**).

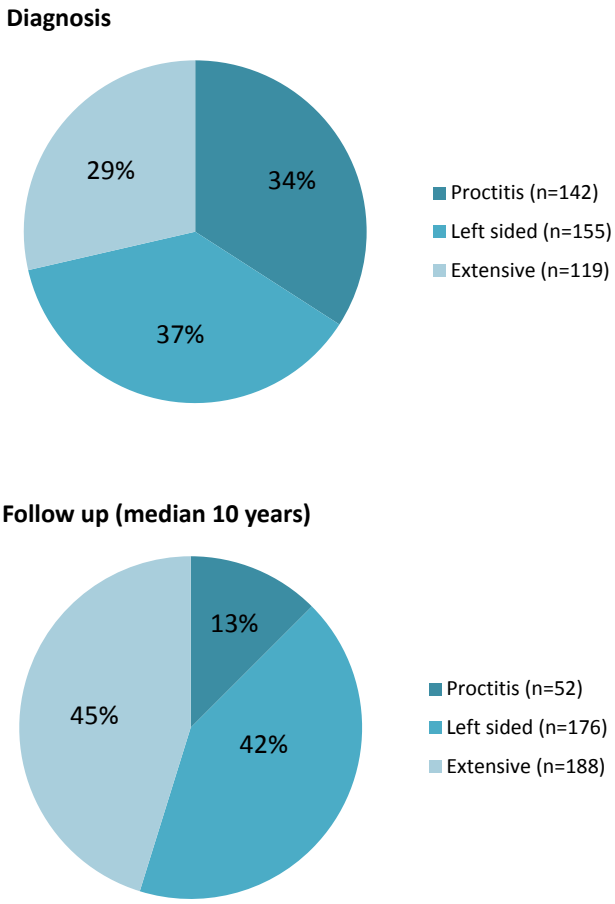


Figure 3. Extent of disease at diagnosis and during follow up (n=416 evaluable patients with colonoscopy data during follow-up)

Disease behaviour

Disease pattern could be evaluated in 378 out of the 506 patients, because of missing data. During follow up, 244 (64.6%) patients showed a disease pattern as shown in figure 1B with less than 1 relapse per year, irrespective of extent of disease at primary diagnosis. Continuously active disease was observed in 36 (9.5%) patients (**Figure 4**).

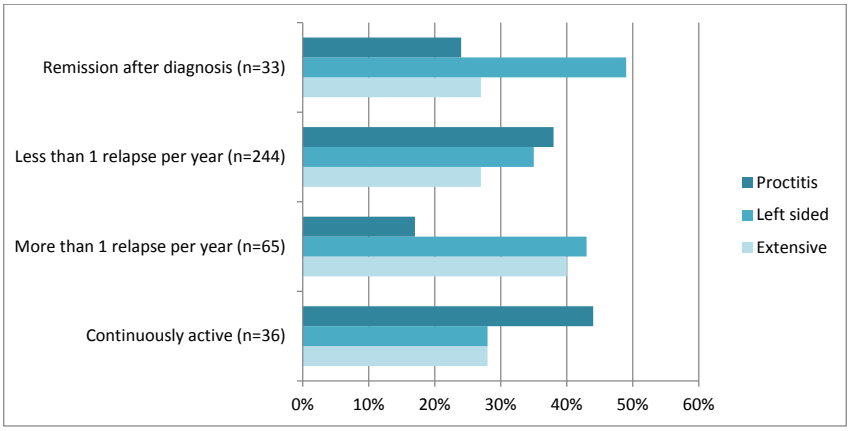


Figure 4. Disease behaviour according to disease extent at diagnosis (n=378 evaluable patients)

Risk factors for proximal disease extension

Univariable analysis identified age at diagnosis, stopped smoking, continuous active disease, systemic steroid treatment, topical steroid treatment, immunomodulators, and biologicals for multivariable regression analysis, based on a p-value of <0.2. Multivariable analysis identified younger age at diagnosis (HR 0.98, 95%CI 0.96 – 0.99) and continuous active disease (HR 2.18, 95%CI 1.27 – 3.73) as independent risk factors for disease extension (**Table 2**).

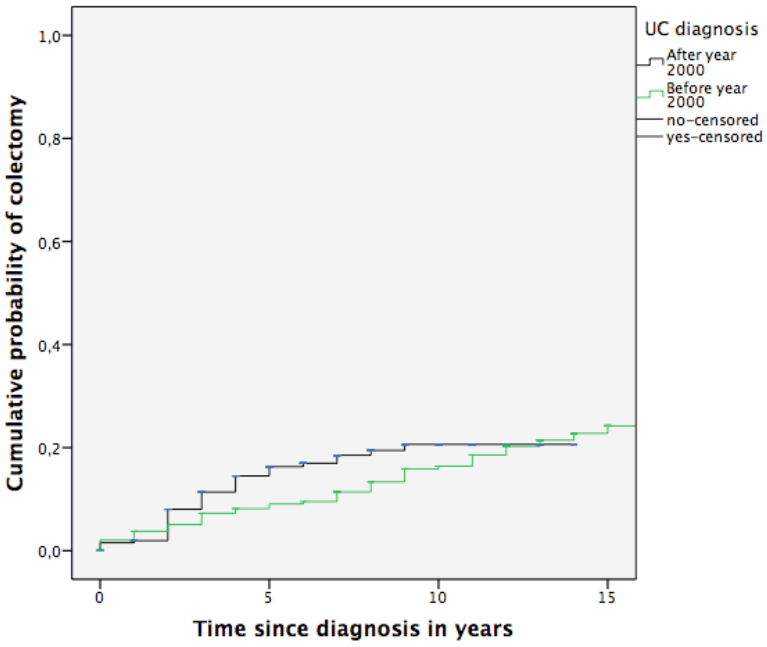
Table 2. Univariable and multivariable cox regression analysis for the risk of proximal disease extension in UC

	Univariable cox analysis HR (95%CI)	P value	Multivariable cox analysis HR (95%CI)	P value
Male sex	0.94 (0.66 – 1.35)	0.742		
Age at diagnosis	0.97 (0.96 – 0.98)	<0.001	0.98 (0.96 – 0.99)	0.004
Current smoking	0.79 (0.46 – 1.35)	0.390		
Stopped smoking	0.74 (0.48 – 1.15)	0.182	(-)	
EIM	1.06 (0.60 – 1.85)	0.843		
PSC	1.26 (0.40 – 3.99)	0.690		
Diabetes	0.94 (0.30 – 2.95)	0.912		
Continuous active disease	2.36 (1.38 – 4.02)	0.002	2.18 (1.27 – 3.73)	0.005
Systemic steroids	1.01 (1.01 – 1.04)	0.001	(-)	
Topical steroids	1.40 (0.95 – 2.06)	0.090	(-)	
5-ASA treatment	1.12 (0.35 – 3.52)	0.852		
Immunomodulators	2.19 (1.49 – 3.20)	<0.001	(-)	
Cyclosporine	1.00 (0.89 – 1.01)	0.483	(-)	
Biologicals	1.65 (1.10 – 2.53)	0.021	(-)	

(-) Indicates a not significant variable that was excluded from multivariable analysis after forward stepwise regression
EIM: extra-intestinal manifestation, PSC: primary sclerosing cholangitis

Risk factors for colectomy

In total, 95 (18.8%) of 506 patients underwent colectomy during follow up with a median time to colectomy of 54 months (IQR 31 – 111). After 5, 10 and 15 years, the cumulative risk of colectomy was 11.7% (95%CI, 8.8 – 14.6), 17.2% (95%CI, 13.5 - 20.7) and 20.8% (95%CI, 16.8 – 25.2), respectively. The cumulative risk of colectomy did not change over time between patients diagnosed before and after the year 2000 (p=0.34) (**Figure 5**). Univariable analysis identified the following variables for multivariable analysis (p<0.2): age at diagnosis, stopped smoking, proctitis at diagnosis, extensive colitis at diagnosis, proximal extension over time, continuous disease activity, systemic steroids, topical steroids, 5-ASA treatment, immunomodulators, cyclosporine and biologicals. Multivariable analysis identified continuously active disease (HR 7.05, 95% CI 4.23 – 11.77), systemic steroids (HR 3.25, 95% CI 1.37 – 7.71) and cyclosporine treatment (HR 2.80, 95% CI 1.66 – 4.72) as independent risk factors for colectomy, whereas proctitis at diagnosis (HR 0.43, 95% CI 0.22 – 0.86) carried a lower risk of colectomy (**Table 3**).



Year	0	5	10	15
No. at risk				
Before 2000	244	218	190	138
After 2000	262	167	71	0

Figure 5. Kaplan Meier curve for the cumulative risk of colectomy before and after the year 2000

Table 3. Univariable and multivariable cox regression analysis for the risk of colectomy in UC

	Univariable cox analysis HR (95%CI)	P value	Multivariable cox analysis HR (95%CI)	P value
Male sex	1.04 (0.68 – 1.59)	0.867		
Age at diagnosis	0.98 (0.97 – 1.00)	0.069	(-)	
Current smoking	0.74 (0.36 – 1.52)	0.409		
Stopped smoking	0.55 (0.27 – 1.10)	0.091	(-)	
EIM	0.77 (0.37 – 1.62)	0.495		
PSC	1.44 (0.45 – 4.57)	0.541		
Diabetes	0.05 (0.01 – 55.2)	0.399		
Proctitis*	0.44 (0.23 – 0.84)	0.014	0.43 (0.22 – 0.86)	0.016
Left sided colitis*	1.32 (0.78 – 2.23)	0.305		
Extensive colitis*	1.56 (0.90 – 2.70)	0.111	(-)	
Proximal extension	1.75 (0.87 – 3.52)	0.118	(-)	
Continuous active disease	10.7 (6.51 – 17.4)	<0.001	7.05 (4.23 – 11.77)	<0.001
Systemic steroids	3.52 (1.86 – 6.66)	<0.001	3.25 (1.37 – 7.71)	0.008
Topical steroids	1.41 (0.84 – 2.39)	0.198	(-)	
5-ASA treatment	0.28 (0.12 – 0.65)	0.003	(-)	
Immunomodulators	2.63 (1.51 – 4.58)	0.001	(-)	
Cyclosporine	4.61 (2.84 – 7.48)	<0.001	2.80 (1.66 – 4.72)	<0.001
Biologicals	2.98 (1.86 – 4.78)	<0.001	(-)	

* Disease extent at diagnosis
(-) Indicates a not significant variable that was excluded from multivariable analysis after forward step-wise regression
EIM: extra-intestinal manifestation, PSC: primary sclerosing cholangitis

DISCUSSION

In this historical cohort of incident UC cases at a single centre in The Netherlands, the disease extended towards the proximal colon in one third of the patients with initial distal colitis during 10 years of follow up. However, this disease extension was not associated with a higher incidence of colectomy. Conversely, continuous disease activity, use of systemic glucocorticosteroids and cyclosporine treatment were independent risk factors for colectomy. Furthermore, the cumulative colectomy rates remained rather high and did not change over time despite the introduction of biologicals.

Over the past few decades, studies have shown variable rates of disease location at diagnosis in UC. Studies published in the nineties demonstrated that the majority of patients were diagnosed with either proctitis or extensive colitis and only a minority with left sided colitis.[4,5] It is however questionable whether this is a true reflection of the disease distribution

since there was no validated classification system such as the Montréal classification at that time.[9] Furthermore, it may have been more difficult to identify the most proximal extent of inflammation with less advanced imaging techniques and somewhat more restricted access to endoscopy. Another explanation could be that disease behaviour has changed over time due to different treatment policies. In this study, we found that left sided disease was most prevalent at diagnosis and extensive disease at the end of follow-up, which parallels the findings in more recent studies.[2,6]

So far, limited data are available about UC disease behaviour patterns by describing and classifying the frequency and duration of disease relapses during a patient's disease history. The IBSEN study prospectively evaluated disease behaviour in 454 UC patients with a follow up of 5 years, based on the patient's experience and a colonoscopy at diagnosis and 5 years later.[10] The study showed that 59% of the patients experienced a decline in the severity of intestinal symptoms, 22% had a relapse free disease course and 9% had chronic active disease. The present study showed a similar outcome based on objective measures such as the number of relapses and treatment initiation or dose escalation. In addition, we demonstrated that patients with chronic continuous disease have a 2-fold increased risk for proximal disease extension and an 8-fold increased risk for colectomy. Therefore, it is of importance to identify the dynamic nature of the disease for prognosis and monitoring, which could be classified as shown in figure 1.

This study showed that approximately one third of the patients with distal colitis had progression to left sided or extensive colitis during 10 years of follow up. This rate of proximal disease extension is comparable to recently published studies by Solberg et al. and Vester-Andersson et al.[2,6] Interestingly, Solberg et al. showed that patients with proximal disease extension had a higher colectomy rate (28%) compared to those with extensive colitis at diagnosis (19%). This may reflect a more aggressive phenotype in these patients. In the present study, younger patients and patients with continuous active disease appeared to be the only independent predictors for proximal disease extension. However, we were not able to find a significant association between proximal disease extension and colectomy.

Over the past few decades, the published colectomy rates in UC have been declining.[11] Earlier studies reported colectomy rates as high as 25% at 10 years.[4,5] A recent Scandinavian study showed a 9-year cumulative colectomy rate of 14.5% in the early eighties that decreased to 9.1% after the year 2003.[12] A large European multicentre study showed an overall 10-year cumulative colectomy rate of 8.7% even though one of the collaborating centres (the Copenhagen centre) had a much higher colectomy rate (25.7% at 10 years) than the other participating centres.[13] The present study however showed a cumulative risk of colectomy of 17.2% at 10 years, which was more comparable to the data from Copenhagen. Of course, the high colectomy rate can be explained by significant referral bias. Patients with more severe and refractory UC are often referred to an academic institution. In line with a

previous Dutch population based study[14], our study showed that the cumulative colectomy rate did not decrease over time. However, the median time until colectomy was longer than previously described (4,5 years versus approximately 2 years).[10] This finding suggests that the advent of biologicals may not prevent but rather delay colectomy in the patients with more severe disease.

An important limitation of this study is the retrospective design, which is always prone to missing data. Colonoscopy was only performed on indication and therefore unavailable in some of the patients. Furthermore, some patients have been lost to follow up or referred back to a primary/secondary care setting. Therefore, there may have been an overrepresentation of patients with more severe disease. The strength of the present study is that it is the first cohort in which immunomodulators and biologicals were commonly used. Furthermore, this study included a large cohort with a long follow up period that solely focused on UC patients.

In conclusion, in this cohort we found that one third of the patients with UC have inflammation that extends more proximally over time. Younger age at diagnosis and continuous active disease were independent risk factors for proximal disease extension. However, proximal disease extension was not a risk factor for colectomy, but this risk was rather associated with continuously active disease, systemic steroids and cyclosporine treatment. The cumulative colectomy rates have remained high over time despite the introduction of biologicals.

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CHAPTER 6

Comparison of health-related quality of life and disability in ulcerative colitis patients following restorative proctocolectomy with ileal pouch-anal anastomosis versus anti-tumor necrosis factor therapy

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ABSTRACT

Background and aims: Health related quality of life (HRQL) and disability were compared in ulcerative colitis (UC) patients who underwent proctocolectomy with ileal pouch-anal anastomosis versus patients who receive(d) treatment with anti-Tumor Necrosis Factor (anti-TNF) agents.

Methods: UC patients who underwent restorative proctocolectomy or started anti-TNF treatment between January 2010 and January 2015 were included at two tertiary referral centers. A matched cohort was created using propensity score matching for the covariates disease duration, Montreal classification, age and gender. Disease specific HRQL and disability were assessed using the COREFO score and IBD Disability Index and generic HRQL was evaluated using EQ-5D-3L and SF-36 questionnaires.

Results: In total, 297 patients were included of whom 205 patients (69.0%) responded. Fifty-nine pouch patients were matched to 59 anti-TNF treated patients. Pouch patients reported better general health scores ($p=0.042$) compared to the anti-TNF group (SF-36). No differences were found for the EQ-5D-3L and IBD Disability Index between the two groups. Pouch patients had significant higher COREFO subscores compared to anti-TNF treated patients for “stool frequency” ($p<0.001$), “anti-diarrheal medication use” ($p<0.001$) and “stool related aspects” ($p=0.004$), of which the latter was due to more peri-anal skin irritation problems ($p<0.001$).

Conclusions: UC patients who underwent restorative proctocolectomy reported higher stool frequencies and peri-anal skin irritation problems compared to anti-TNF treated subjects, but this did not affect overall disease specific disability outcomes. On the other hand, patients in the surgery group reported better outcomes for generic health perspective compared to those in the anti-TNF group.

INTRODUCTION

Ulcerative colitis (UC) has a significant impact on health related quality of life (HRQL), even during quiescent disease phases.[1,2] According to current guidelines, management of moderate to severe UC consists of biological therapy or surgical interventions, most frequently used in a step-up approach. Two randomized placebo-controlled trials with infliximab showed that UC patients had a substantially improved HRQL after one year of this treatment compared to patients treated with placebo.[3] However, the clinical use of anti-TNF agents is hampered by primary and secondary loss of response in up to 55 % of the patients, as well as by potentially serious side effects.[4]

Restorative proctocolectomy with ileal pouch-anal anastomosis is the treatment of choice for patients with therapy refractory UC and provides satisfactory long-term outcomes in the majority of patients. A prospective observational study in 391 UC patients who underwent restorative proctocolectomy showed that, after five years, 72% of the patients was fully continent, 81% judged their quality of life as much better compared to the situation before surgery and 96% judged their overall satisfaction as excellent or good.[5] However, restorative proctocolectomy is associated with short and long-term complications including acute or chronic pouchitis, with an incidence varying between 16 and 48%, and pouch failure, with an incidence varying between 3 and 30%.[6]

In certain countries, restorative proctocolectomy is considered a cheaper and equally effective treatment option compared to anti-TNF therapy in UC patients. As a consequence, reimbursement of anti-TNF maintenance treatment is limited. HRQL and disability outcomes are important outcome parameters that can be used to compare the relative value of different therapeutic strategies. Up to now, few studies are available that compared HRQL and disability outcomes in UC patients that underwent restorative proctocolectomy and those that receive treatment with anti-TNF agents. Although restorative proctocolectomy is generally accepted as being a sequential option after anti-TNF treatment, it is of important interest to compare these groups concerning quality of life standards. If HRQL and disability scores would be significantly better in UC patients in the surgery group compared to UC patients in the anti-TNF group, one might consider restorative proctocolectomy as a possible option in an earlier stage of the disease, instead of seeing it as an 'end-of-the-line' option until medical treatment is exhausted.

We recently studied HRQL and disability outcomes in a selected group of UC patients who had an optimal response to anti-TNF treatment (i.e. with mucosal healing and absence of side effects) and after restorative proctocolectomy.[7] No differences in HRQL and disability were found, except for stool frequency and anti-diarrheal medication use that was significantly higher in the surgery group. The aim of the present study was to explore HRQL and disability in a larger, representative cohort of patients with moderate to severe UC who

receive(d) anti-TNF treatment (including patients with side effects or loss of response to these agents) and patients that underwent restorative proctocolectomy (including patients with post-operative complications and/or pouchitis).

MATERIALS AND METHODS

Study design

This retrospective cohort study was performed at two tertiary IBD referral centers: the Academic Medical Center in Amsterdam, The Netherlands and the University Hospitals in Leuven, Belgium. All patients with moderate to severe UC diagnosed by endoscopy and confirmed by histopathology who started treatment with an anti-TNF agent (infliximab, adalimumab or golimumab) and who received at least induction treatment (i.e. 3 infusions with infliximab at week 0-2-6; 2 injections with adalimumab at week 0 and 2; or 2 injections with golimumab at week 0 and 2) between January 1st 2010 and January 1st 2015 constituted the anti-TNF group. Patients who underwent restorative proctocolectomy with ileal pouch-anal anastomosis and ileostomy closure between January 1st 2010 and January 1st 2015 were approached for the surgery group. Patients under 18 years of age at the start of the intervention (i.e. (procto)colectomy or start with anti-TNF therapy) were excluded. Patients that underwent (procto)colectomy for other indications than for active refractory UC (i.e. dysplasia/cancer, iatrogenic perforation during colonoscopy, etc.) were also excluded. Treatment failure in the anti-TNF group was defined as anti-TNF treatment cessation due to primary or secondary loss of response. These patients did not undergo a (procto)colectomy (yet), Treatment failure in the surgery group was defined as pouch excision or placement of a permanent ileostomy.

Ethical considerations

The institutional review board of the Academic Medical Center in Amsterdam granted a waiver for this study.

Outcome measures

Primary outcome measures were generic HRQL and disability and disease specific HRQL and disability. Generic HRQL was measured with the EuroQol 5D-3L (EQ-5D-3L) questionnaire and the Short Form health survey 36 (SF-36). Both questionnaires are commonly used HRQL questionnaires in medical research with high validity and reliability in functional status, well-being and general perception of health.[8,9] The EQ-5D-3L consists of 5 subscales and a self-rated health score (Visual Analogue Scale (VAS) score) ranging from 0 to 100. The five subscales include mobility, self-care, daily activities, pain or discomfort, and anxiety

or depression, which are scored as 1 (no problems), 2 (moderate problems) or 3 (severe problems). The EQ-5D-3L scale scores were dichotomized into no problems and problems (including moderate to severe problems). This is advised when the number of reported level 3 problems are low, which is the case in UC patients.[8]

The SF-36 contains 8 subscales: i.e. physical functioning, role limitations due to physical functioning, role limitations due to emotional functioning, bodily pain, general health, vitality, social functioning, and mental health. SF-36 scale scores were linearly transformed into scores ranging from 0 to 100, with a higher score indicating better functioning (i.e. better quality of life).[10] Disease specific HRQL and disability were measured with the Colorectal Functional Outcome (COREFO) questionnaire and the Inflammatory Bowel Disease Disability Index (IBD-DI). The COREFO questionnaire focusses on stool related aspects and the influence on daily activities and includes 5 subscales: i.e. fecal incontinence, social impact, stool frequency, stool related aspects (e.g. peri-anal skin irritation, pain during bowel movements and rectal blood loss) and the need for anti-diarrheal medication use.[11] The recently validated IBD-DI questionnaire measures disease specific disability in daily life in IBD patients.[12] The total IBD-DI score and the COREFO scale scores were linearly transformed into a score ranging from 0 to 100, with higher scores indicating more severe problems (i.e. impaired quality of life).

Secondary outcome measures were defined as post-operative complications after restorative proctocolectomy and adverse events related to anti-TNF treatment. Post-operative complications included cuffitis (diagnosed by endoscopy), acute pouchitis (confirmed by endoscopy and antibiotic treatment lasting not longer than 4 weeks), chronic pouchitis (confirmed by endoscopy and antibiotic treatment lasting longer than 4 weeks), anastomotic leakage or abdominal abscess (confirmed by imaging), small bowel obstruction (confirmed by imaging), anastomotic stricture (with requirement of endoscopic balloon dilatation), pouch fistula (confirmed by endoscopy and/or imaging), fecal incontinence and sexual dysfunction (such as impotence, retrograde ejaculation or dyspareunia). Adverse events due to anti-TNF treatment included acute (immediate) infusion reactions (hypo- or hypertension, dyspnea, fever and/or chest pain), delayed infusion reactions (arthralgia and/or myalgia), serious infections (pneumonia, sepsis, fungal, viral and bacterial infections and tuberculosis), skin reactions (eczematiform or psoriasiform lesions), malignancies (such as melanoma and lymphoma), demyelinating neurological disease and heart failure.

Procedure

Eligible patients were identified by electronic search of patient records. Patient characteristics were collected by chart review. Patients were invited by email or letter to complete the paper based questionnaires. The questionnaires comprised the four validated questionnaires (EQ-

5D-3L, SF-36, COREFO and IBD-DI) and a general questionnaire including smoking habits, race etc. If patients did not respond within two weeks, a second questionnaire was sent by email or regular post. If patients did not respond within the next two weeks, they were contacted by phone or email.

Statistical analysis

Categorical data are presented as frequencies and percentages. Continuous data are presented as mean with standard deviation (SD) or median with interquartile range [IQR] according to distribution. Chi-square test or Fisher's exact test were used to compare categorical variables. Independent samples T-Test was used to compare normally distributed variables and the Mann-Whitney U test for non-parametric data. Propensity score matching was used to form matched sets which share a similar value of the propensity score. The propensity score describes the probability of the treatment assignment, with the intention to mimic some of the particular characteristics of a randomized controlled trial.[13] The covariates disease duration and Montreal classification were used as disease specific covariates and gender and age as general covariates. A nearest-neighbour matching algorithm was used with caliper width equal to 0.2 of the standard deviation of the logit of the propensity score. Variables with a two-sided p-value of less than 0.05 were considered significant. All statistical analyses were performed using IBM® SPSS® for Windows® version 22 (IBM Corp., Armonk, NY, United States). For propensity-score matching the SPSS R-Plugin with requirement of R version 2.15.3 (The R Foundation for Statistical Computing 2013©) and IBM® SPSS® Statistics - Essentials for R 22 were used.

RESULTS

Baseline characteristics

In total 416 UC patients were identified **(figure 1)**, 159 in the surgery group and 255 in the anti-TNF group. In the surgery group 51/159 patients were excluded: 3 patients were lost to follow up, 8 patients were under the age of 18 years at proctocolectomy, 34 patients underwent restorative proctocolectomy for a (pre)malignant lesion and 2 patients for an iatrogenic perforation, and 4 patients were deceased. In the anti-TNF group, 66/255 patients were excluded: 13 patients were lost to follow up, 9 patients were under the age of 18 years at start of the treatment, 32 patients underwent a proctocolectomy, 1 patient did not complete induction therapy and 11 patients were deceased. None of the 32 patients who were excluded for undergoing a proctocolectomy were included in the surgery group. Eleven (3.7%) patients declined participation and 81 (27.3%) did not respond.

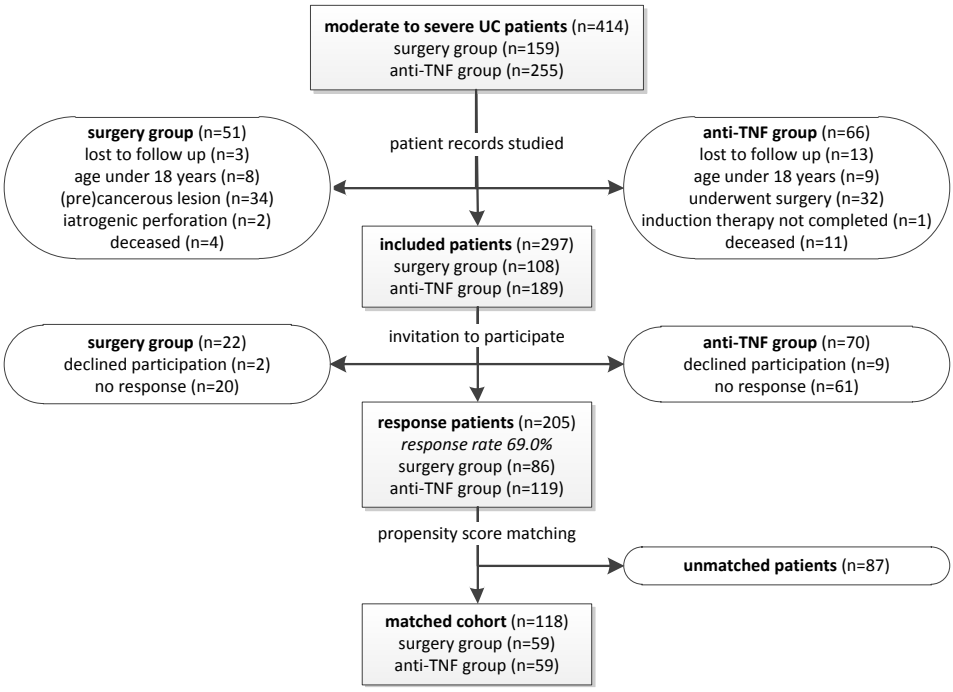

Figure 1. Flowchart of included and excluded patients. Anti-TNF, anti-tumor necrosis factor; UC, ulcerative colitis.

After two invitations, 205 (69.0%) patients completed the questionnaires. After propensity score matching, the matched cohort consisted of 59 pouch patients and 59 anti-TNF treated patients.

Patients in the surgery group had significantly more often (documented) severe endoscopic disease (defined as a Mayo endoscopic subscore of 3) prior to their surgery compared to anti-TNF treated patients before starting treatment (56.9% vs. 85.1% respectively, p=0.005). They also received significantly more often thiopurines (90.6% vs. 66.1% respectively, p=0.002) and (other) anti-TNF agents (92.5% vs. 30.5%, p<0.001) compared to patients in the anti-TNF group prior to the intervention (i.e. before starting anti-TNF treatment in the anti-TNF group or before undergoing restorative proctocolectomy in the surgery group). Only 1 patient (1.7%) in the surgery group had pouch failure and underwent placement of an ileostomy. In the anti-TNF group, 18 (28.8%) patients stopped treatment due to treatment failure (i.e. loss of response or adverse events). The median [IQR] time between ileostomy closure and completion of the questionnaire was 30.5 [18.0-41.0] months. The overall median [IQR] anti-TNF treatment duration of the last anti-TNF treatment in the anti-TNF group was 20.0 [12.0-35.0] months. The median [IQR] time between initiation of anti-TNF and completion of the questionnaire was 29.0 [20.0-49.0] months. The baseline characteristics are depicted in **table 1**.

Table 1 Baseline characteristics matched cohort

	Surgery group (*n*=59)	Anti-TNF group (*n*=59)	*p*-value
Demographic characteristics			
Female gender (%)	32/59 (54.2)	25/59 (42.4)	0.197
Age, years (SD)	45.8 ± 12.4	40.9 ± 14.8	0.058
Caucasian (%)	52/58 (89.7)	53/59 (89.8)	0.975
Current smoking (%)	5/59 (8.5)	3/59 (5.1)	0.717
Clinical characteristics			
Disease duration, months [IQR]	66.5 [28.8-146.0]	52.0 [22.0-156.0]	0.406
Extraintestinal manifestations[1] (%)	2/59 (3.4)	6/59 (10.2)	0.272
Montreal classification[2] (%)			1.000
Proctitis	2/59 (3.4)	2/59 (3.4)	
Left sided colitis	14/59 (23.7)	14/59 (23.7)	
Pancolitis	43/59 (72.9)	43/59 (72.9)	
Endoscopic Mayo score[2] (%)			0.005*
Mild/no disease	2/47 (4.3)	2/51 (4.3)	
Moderate disease	5/47 (10.6)	20/51 (39.2)	
Severe disease	40/47 (85.1)	29/51 (56.9)	
Comorbidity present[3](%)	18/59 (30.5)	24/59 (40.7)	0.249
Treatment-related characteristics			
History of appendectomy (%)	8/59 (13.6)	6/59 (10.2)	0.569
History of other abdominal surgery[4] (%)	8/59 (13.6)	11/59 (18.6)	0.452
Age at start of treatment, years[5] (SD)	42.9 ± 12.1	38.2 ± 14.7	0.064
Time since start treatment, months[6] [IQR]	30.5 [18.0-41.0]	29.0 [20.0-49.0]	0.363
Treatment failure (%)	1/59 (1.7)	18/59 (30.5)	<0.001*
Anti-TNF treatment duration, months [IQR]	*n.a.*	20.0 [12.0-35.0]	*n.a.*
Prior medication[2] (%)			
5-ASA topical	37/53 (69.8)	40/59 (67.8)	0.818
5-ASA systemic	47/53 (88.7)	55/59 (93.2)	0,513
Corticosteroids topical	16/59 (27.1)	20/53 (37.7)	0.230
Corticosteroids systemic	58/59 (98.3)	53/53 (100.0)	1.000
Thiopurines	48/53 (90.6)	39/59 (66.1)	0.002*
Cyclosporine	7/53 (13.2)	5/59 (8.5)	0,419
Anti-TNF agents	49/53 (92.5)	18/59 (30.5)	<0.001*
Infliximab	49/53 (92.5)	14/59 (23.7)	<0.001*
Adalimumab	13/53 (24.5)	6/59 (10.2)	0.043 *
Golimumab	0/59 (0.0)	0/59 (0.0)	*n.a.*
Current medication[7] (%)			
5-ASA topical	2/59 (3.4)	3/59 (5.1)	1.000
5-ASA systemic	0/59 (0.0)	30/59 (50.8)	<0.001*
Corticosteroids topical	2/59 (3.4)	2/59 (3.4)	1.000
Corticosteroids systemic	0/59 (0.0)	7/59 (11.9)	0.013*
Infliximab	0/59 (0.0)	29/59 (49.2)	<0.001*
Adalimumab	0/59 (0.0)	8/59 (13.6)	0.006*
Golimumab	0/59 (0.0)	4/59 (6.8)	0.119
Thiopurines	0/59 (0.0)	18/59 (30.5)	<0.001*
Other[8]	5/59 (3.4)	10/59 (16.9)	0.029*

SD, standard deviation; IQR, interquartile range; * statistical significant value (p<0.05). [1]Including arthritis, erythema nodosum, pyoderma gangrenosum and uveitis. [2]Prior to start of the treatment. [3]At least one comorbidity present including autoimmune disease (e.g. reumatoid arthritis), cardiovascular disease (e.g. myocardinfaction), pulmonal disease (e.g. COPD), depression or anxiety disorders, DMII, hypertension, PSC or neurological disease (epilepsy). [4]Including abdominal hernia correction, partial colectomy, cholecystectomy, Caesarean section, hysterectomy, liver transplantation. [5]Age at (procto)colectomy or at the start of anti-TNF treatment. [6]Time between ileostomy closure after pouch surgery or anti-TNF initiation and completion of the questionnaire. [7]At the day of completion of the questionnaire. [8]Including vedolizumab, etrolizumab, tofacitinib, tacrolimus, PF-00547659, curcumin and AMG-181

Generic and health related quality of life

No significant differences were found between the two groups in the percentage of reported problems regarding the EQ-5D-3L questionnaire for the scale scores mobility, self-care, daily activities, pain or discomfort and anxiety or depression **(figure 2)**. Likewise, no difference was found in the median [IQR] EQ-5D self-rated health score between the surgery and the anti-TNF group (75.0 [70.0-85.0] vs. 75.0 [70.0-80.0] respectively, p=0.771) **(figure 3)**. Patients in the surgery group had significantly higher median [IQR] scores for the SF-36 general health score, indicating a better health status, compared to anti-TNF treated patients (55.0 [40.0-70.0] vs. 45.0 [40.0-55.0] respectively, p=0.042) **(figure 4)**. This higher general health score was due to a greater proportion of patients in the surgery group who agreed with the statements: "I am as healthy as anybody I know" (47.5% vs. 25.9%, p=0.047) and "My health is excellent" (61.0% vs. 32.7%, p=0.003) compared to patients in the anti-TNF group. No statistical significant differences were observed between the two groups regarding the other SF-36 items, such as mental health, role limitations because of physical or emotional problems, bodily pain, social functioning and vitality.

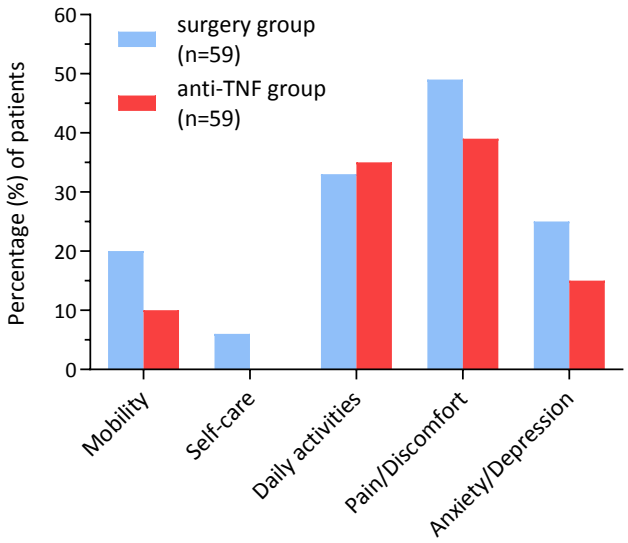

Figure 2. Percentage (%) of patients who reported problems for each subscale of the EQ-5D-3L Questionnaire. Mobility: p=0.124, self-care: p=0.119, daily activities: p=0.847, pain or discomfort: p=0.266, anxiety or depression: p=0.170

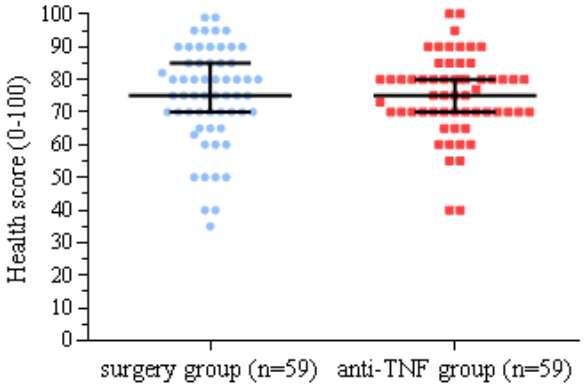

Figure 3. Median [IQR] Self-rated health scores (VAS-score) for the EQ-5D-3L questionnaire (p=0.771)

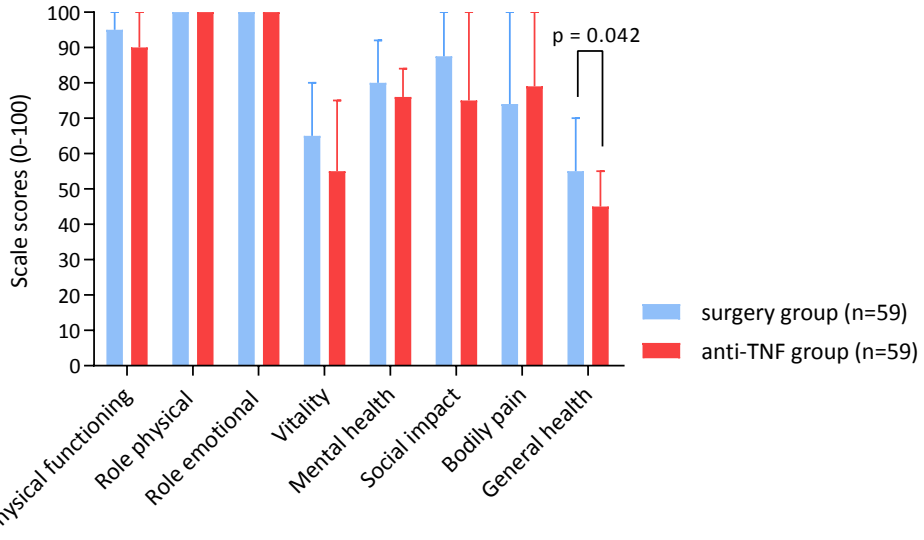

Figure 4. Median [IQR] scale scores for the SF-36 questionnaire. Physical functioning: p=0.600, role limitations due to physical functioning; p=0.774, role limitations due to emotional functioning: p=0.348, vitality: p=0.167, mental health: p=0.054, social functioning: p=0.720, bodily pain: p=0.378, general health: p=0.042

Disease specific HRQL and disability

Pouch patients had significantly higher median [IQR] scores on the COREFO questionnaire (indicating more problems) compared to anti-TNF treated patients for stool frequency (37.5 [37.5-50.0] vs. 12.5 [12.5-12.5], p<0.001), anti-diarrheal medication use (33.3 [16.7-66.7] vs. 0.0 [0.0-33.3], p<0.001), and stool related aspects (25.0 [8.3-41.7] vs. 8.3 [0.0-16.7], p=0.004) **(figure 5)**. The high median [IQR] scale score for stool related aspects in the surgery

group was due to a higher percentage of patients who reported problems with peri-anal skin irritation (82.8% vs. 37.3% in the surgery and anti-TNF group respectively, p<0.001), whereas the other 2 items in this subscale (i.e. pain during defecation and presence of blood in stools) were not significantly different between the two groups. No differences were found in median [IQR] scale scores for stool related aspects comparing patients in the surgery group with a pouch existing for less than one year to patients with a pouch existing for more than one year (8.3 [0.0-35.4] vs. 15.7 [2.1-33.3] respectively, p=0.441). No significant differences were found between the surgery and anti-TNF group for the median [IQR] total IBD–DI score (17.9 [8.9-28.6] vs. 21.4 [14.3-35.7], p=0.261) **(figure 6)**.

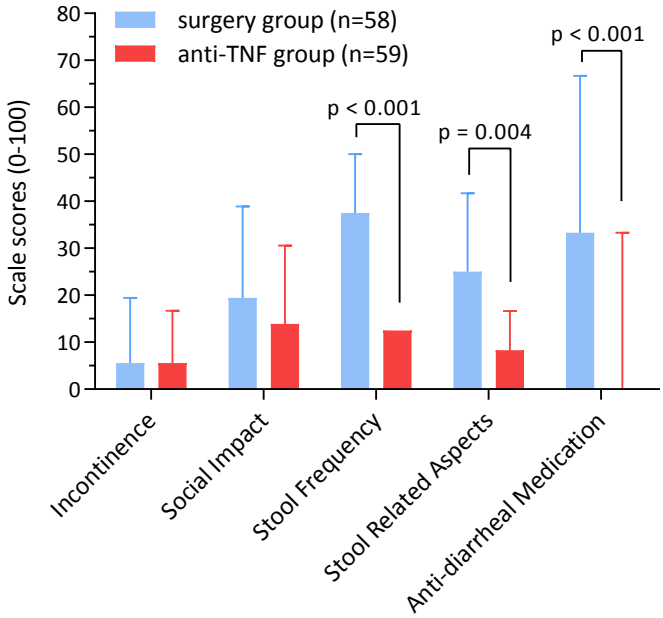

Figure 5. Median [IQR] scale scores for the COREFO questionnaire. Incontinence: p= 0.496, social impact: p=0.137, stool frequency: p<0.001, stool related aspects p=0.004, anti-diarrheal medication use: p<0.001

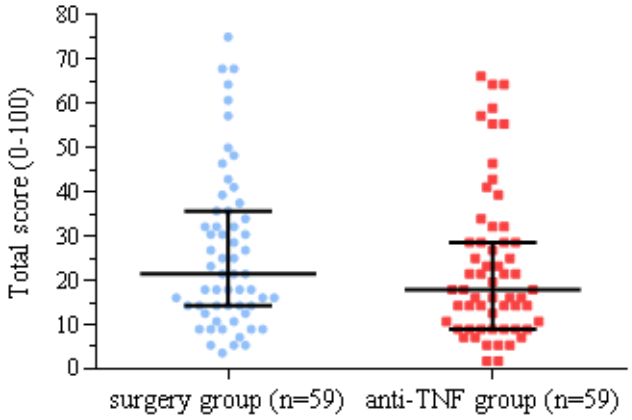

Figure 6. Median [IQR] total scores for the IBD Disability Index (p=0.261)

Post-operative complications and adverse events

In the surgery group, 10 patients (16.9%) had at least one episode of acute pouchitis and 4 patients (6.8%) met the criteria for chronic pouchitis. Other reported post-operative complications were cuffitis (7 patients, 11.9%), small bowel obstruction (1 patient, 1.7%), anastomotic leakage (2 patients, 3.4%), pelvic abscess (1 patient, 1.7%), anastomotic stricture (5 patients, 8.5%) and dyspareunia (1 patient, 1.7%). In 6 patients (10.2%) other complications were reported, including deep venous thrombosis and portal vein thrombosis, post-operative bleeding, ileus and severe abdominal pain requiring prolonged analgesic treatment.

The most frequent reported adverse events in the anti-TNF group were delayed infusion reactions (10 patients, 10.2%), eczematiform or psoriasiform lesions (10 patients, 16.9%), fatigue (11 patients, 18.6%) and other adverse events (10 patients, 16.9%), including (mild) infections, headache and xerosis cutis. One patient (1.7%) was diagnosed with a melanoma during anti-TNF treatment and received curative treatment. Acute infusion reactions, serious infections (such as pneumonia or opportunistic infections), demyelinating diseases or heart failure were not reported.

DISCUSSION

This study compared HRQL and disability outcomes in UC patients who received treatment with restorative proctocolectomy and anti-TNF agents. An unselected group of UC patients was studied, irrespective of their outcomes after surgery or their response to anti-TNF treatment, in an attempt to reflect daily practice. Although a higher frequency of disease specific problems was detected in patients that underwent restorative proctocolectomy,

such as stool frequency, anti-diarrheal medication use and stool related aspects, no significant differences were found with regard to the total IBD-DI score. On the other hand, patients in the surgery group reported better general health perspectives compared to patients in the anti-TNF group, due to a higher rate of agreement with statements "I am as healthy as anybody I know" and "My health is excellent". So-called 'response shift' could be a possible explanation for the better outcome in general health perspective in the surgery group.[14] This implies a change in internal standards, values or conceptualization with changes in the meaning of quality of life after a life-threatening condition or following a long period of chronic disease. Hence, patients who underwent restorative proctocolectomy may rate their current HRQL relatively high compared to the poor HRQL they experienced prior to surgery. Moreover, patients in the surgery group may have experienced surgery as a final solution (they might consider surgery as a 'curative' treatment option) which could affect their perspectives. Last but not least, patients in general dislike to use medication. All these factors may influence the patient's perspective and, as a result, HRQL and disability outcomes in the two groups.

The high(er) stool frequency and anti-diarrheal medication use in the surgery group is likely to be explained by anatomical changes in colectomized patients which is in accordance with previous reports.[7] In this study, significant more stool related problems in the surgery group compared to the anti-TNF group were found, which was mainly due to more peri-anal skin irritation problems. No differences were found in stool related problems comparing surgery patients with a pouch existing for less than one year compared to patients with a pouch existing for more than one year. A recent study consisting of 191 UC patients with a pouch showed that 132 patients (70%) experienced perianal soreness and in 29 out of 132 patients (15%) this was a chronic ongoing problem.[15] In that particular study only 6 patients (3%) of the total surgery group reported their condition as being worse or unchanged after surgery using a VAS-score. In accordance to this, in this study no differences were found in the total IBD-DI score between the surgery and anti-TNF group, indicating that the higher frequency of stool related problems in the surgery group did not affect overall disability. Another study compared HRQL in UC patients who received treatment with anti-TNF agents versus patients that underwent surgery (pouch reconstruction or ileostomy) using the inflammatory bowel disease questionnaire (IBDQ) and found no significant differences in IBDQ scores. However, pouch patients had higher (indicating better) quality-adjusted life years than anti-TNF treated patients due to significantly higher healthcare costs as well as higher productivity loss in anti-TNF treated patients.[16]

In the present study, better general health perspectives in pouch patients were observed using the SF-36 questionnaire. This finding was not detected in the previous study.[7] This contrasting finding is likely explained by the differences in patient populations between both studies. In contrast to this previous study, now patients who showed loss of response

or toxicity to anti-TNF agents as well as patients who did not have quiescent disease while receiving anti-TNF therapy were also included. Moreover, also patients with an unfavorable disease course after surgery were included, such as patients with post-operative complications and/or pouchitis.

A relative large amount of data was retrieved in this study with a considerable questionnaire response rate (69%) in two high-volume tertiary referral centers, however patients in the anti-TNF group had a higher rate of non-responders (61/255, 23.9%) than surgery patients (20/159, 12.6%). Different validated generic and disease specific HRQL and disability questionnaires were used, including the recently validated IBD-DI.[12] To our knowledge, this is the first study that employed the IBD-DI to compare disease specific HRQL and disability between patients who underwent restorative proctocolectomy versus patients who receive(d) treatment with anti-TNF agents.

In this cohort, 92.5% of patients that underwent surgery failed prior treatment with anti-TNF agents. In contrast, 30.5% of patients in the anti-TNF group failed prior treatment with one anti-TNF agent (23.7% failed on infliximab and 10.2% failed on prior adalimumab treatment). Thus, the majority of patients in the surgery group failed to respond to earlier anti-TNF therapy and they were compared to anti-TNF treated subjects. This might indicate that surgery patients had a more severe UC phenotype than the patients in the anti-TNF group, which obviously may have biased the results. Confounding by indication might be another limiting factor of this non-randomized study, since patient preferences may have biased the results. However, we tried to minimize this risk by creating a matched sample for the covariates disease duration, Montreal classification, age and gender. We were not able to match for disease activity due to the high rate of missing variables for endoscopic mayo score.

Only 1 patient (1.7%) with pouch failure with placement of an ileostomy was reported in this study. A large prospective study of 3707 patients that underwent pouch surgery for different indications reported a pouch failure rate of 5.3%.[17] The ECCO guideline states that there is clear evidence that high volume surgeons in high volume centers achieve lower pouch failure rates.[18] The low incidence of pouch failure in this cohort is probably due to the fact that this study was conducted at two high volume IBD referral centers and this might have beneficially influenced HRQL and disability outcomes of patients in the surgery group. However, patients in the anti-TNF group were also treated in the most optimal conditions by applying therapeutic drug monitoring in the majority of the cases. Important to note is the relative low incidence of reported pouchitis (16.9 percent acute pouchitis and 6.8 percent chronic pouchitis), since the reported incidence of pouchitis in prior studies varies between 16 and 48 percent.[6] Also notable is the absence of reported acute infusion reactions. A retrospective study in Crohn's disease patients reported an overall incidence of acute infusion reactions to infliximab of 5.4%, affecting 8.4% of the patients.[19] This difference

might be explained by the predetermined strict definition of an acute infusion reaction, which comprised mainly severe reactions (e.g. hypotension, chest pain etc.), and secondly by the retrospective character of the study with possible underreporting of symptoms in the patient records.

In conclusion, this study showed that UC patients who underwent restorative proctocolectomy had a significantly higher stool frequency, used more anti-diarrheal medication and had more peri-anal skin irritation problems when compared to anti-TNF treated patients. However, these findings did not influence overall disease specific disability outcomes. Interestingly, pouch patients reported better general health perspectives, which can be attributed to the presumed 'curative' character of surgery in UC and a phenomenon called responsive shift. This study provides applicable information to guide patients in clinical decision-making and may serve as basis for cost-effectiveness studies. Future prospective longitudinal studies may be beneficial in order to obtain more insights with regard to HRQL and disability at different time points during the disease course in these patients.

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PART III

Restorative proctocolectomy and ileal pouch anal anastomosis

CHAPTER 7

A multicentre evaluation of risk factors for anastomotic leakage after restorative proctocolectomy with ileal pouch-anal anastomosis for inflammatory bowel disease

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ABSTRACT

Background: Anastomotic leakage is a major complication after restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA). Identification of patients at high-risk of leakage may influence surgical decision-making. The aim of this study was to identify risk factors associated with anastomotic leakage after restorative proctocolectomy with IPAA.

Methods: Between September 1990 and Jan 2015, patients who underwent IPAA for inflammatory bowel disease were identified from prospectively maintained databases of three tertiary Referral Centres. Retrospective chart review identified additional data on demographic and surgical variables. Multivariable regression models were developed to identify risk factors for anastomotic leakage. Separate analyses were performed for type of procedure.

Results: A total of 640 patients (56.9% male) were included with a median age of 38 years (IQR 29 - 48). Ninety- six (15.0%) patients developed anastomotic leakage. Multivariable regression analysis demonstrated that being overweight (BMI > 25) (OR 1.92; 95%CI 1.15 – 3.18) and ASA score (> 2) (OR 1.91; 95%CI 1.03 – 3.54) were independent risk factors for anastomotic leakage in patients that underwent a completion proctectomy. A disease course of > 5 years (OR 2.34; 95%CI 1.42 – 3.87) and concurrent combination of anti-TNF and steroids (OR 6.40; 95%CI 1.76 – 23.20) were independent risk factors for anastomotic leakage in patients that underwent a proctocolectomy and IPAA.

Conclusions: Independent risk factors for anastomotic leakage in IBD patients undergoing IPAA are BMI >25, ASA score >2, disease course > 5 years, and concurrent steroid and anti-TNF treatment, with a different risk profile for one stage proctocolectomy and completion proctectomy procedures.

INTRODUCTION

Restorative proctocolectomy with ileal pouch anal anastomosis (IPAA) has become the standard surgical procedure in patients with ulcerative colitis (UC), indeterminate colitis, familial adenomatous polyposis (FAP) and selected cases of Crohn's disease.[1] Anastomotic leakage represents a major early complication after IPAA surgery. The incidence of clinically significant leaks has been shown to vary between 5 and 15% and does not seem to decrease over the past decade despite improvement in surgical techniques and perioperative care.[2–4] Anastomotic leakage may ultimately lead to pouch dysfunction or pouch failure with associated negative impact on quality of life.[5–7] In order to prevent or diminish the severity of these complications, most surgeons perform a defunctioning loop ileostomy during the IPAA procedure. However, temporary defunctioning does not abolish the risk of a leak and adds an extra surgical intervention, with its own morbidity.[8] Therefore, selective defunctioning of high-risk patients might be the most appropriate approach, but this would require availability of risk factors with high predictive value.

Various studies have identified risk factors for septic complications after colorectal surgery.[9–12] Studies specifically analysing risk factors for anastomotic leakage after IPAA surgery are limited. In most series addressing this issue, standard defunctioning is performed, and it therefore is questionable whether these results can be directly extrapolated to a one-stage procedure. In addition, most studies are relatively dated and do not take into account the potential influence of anti-TNF treatment. Although the association between preoperative steroid use and anastomotic leakage has become evident, the influence of anti-TNF remains inconclusive.[12–14]

The objective of our multicentre cohort study was therefore to identify pre- and peroperative predictive risk factors associated with anastomotic leakage after IPAA surgery for IBD.

METHODS

Study design

This multicentre cohort study included all eligible IBD patients who underwent IPAA at the Academic Medical Center (AMC) in Amsterdam, The Netherlands, University Hospitals Leuven (UZ Leuven), Belgium, and the Mayo Clinic in Scottsdale Arizona, USA. Included patients from the AMC and UZ Leuven underwent IPAA between September 1990 and Jan 2015. Included patients from Mayo underwent IPAA between March 2009 and July 2012. All three hospitals are tertiary Referral Centres with an average procedure rate of 15-30 IPAA's each year. After consultation with the medical ethical boards, approval was not required because of the observational design.

Patients and Data Collection

Patients who underwent IPAA were identified from prospectively maintained procedural databases and combined for further analysis. All three hospitals maintain an institutional administrative database covering all surgical procedures. A retrospective chart review identified additional data on patient characteristics and surgical variables.

The demographic variables that were extracted were gender, age, smoking habit, body mass index (BMI), weight loss (more than 5% of total body weight in one month before surgery), comorbidity grouped by organ system, and the American Society of Anaesthesiologists classification (ASA score). Preoperative variables included preoperative diagnosis, duration of disease course, steroids, and anti-TNF usage (within 3 months before IPAA creation). Haemoglobin, CRP, and albumin levels were also recorded. Surgical variables included proctocolectomy or completion proctectomy, laparoscopic or open approach, type of pouch created, hand sewn or stapled pouch anal anastomosis, creation of a defunctioning ileostomy, perioperative blood transfusion, duration of surgery and postoperative length of stay.

Overweight was defined as such when the body mass index (BMI) was > 25 kg/m^2. The Charlson comorbidity index (CCI) score was calculated for each individual patient by weighing their comorbidities and adding 1 point for each decade over the age of 40 years.[15] Based on previous literature and clinical relevance, the CCI was dichotomized with a cut-off point of 3, and the ASA score with a cut-off point of 2.[16] Disease duration was defined as the time between diagnosis and pouch surgery. Disease duration of more than 5 years was considered long-term. Perioperative steroid use was defined as patients using more than 20 mg/day within 12 weeks to IPAA. Anti-TNF use was defined as patients using medication within 12 weeks to IPAA procedure. The 12-week cut-off point was chosen based on the anti-TNF half-life.[17]

IPAA procedure

Within all three institutes, experienced colorectal surgeons or a supervised colorectal fellow performed the IPAA procedures. Preoperative mechanical bowel preparation was used for patients treated at Mayo. Patients were treated in an elective setting by a 1-stage procedure being a proctocolectomy with IPAA creation, or a 2-stage procedure, which is a proctocolectomy with IPAA and defunctioning ileostomy, followed by reversal at the second stage. Acute patients were treated by a 3-stage procedure, being a subtotal colectomy with end-ileostomy, followed by completion proctectomy with IPAA and defunctioning ileostomy, and ileostomy reversal at the third stage. In case a defunctioning ileostomy was omitted from the 3-stage procedure, the procedure was called a 'modified' 2-stage. Up until 1995, the open approach was standard practice, therefore only a small percentage of patients underwent a laparoscopic procedure. At the AMC, the hand-assisted laparoscopic IPAAs were

carried out via a Pfannenstiel incision. At UZ Leuven and Mayo, the complete laparoscopic procedures were performed, either by multiport or single incision. At the AMC and UZ Leuven, a defunctioning ileostomy was created at the surgeon's discretion (e.g. medication, severe proctitis, mucosectomy with a hand sewn anastomosis, an incomplete 'doughnut' after stapling, or an anastomosis with high tension). In all 3 hospitals, a pouchography/ endoscopy was performed before ileostomy reversal.

Outcomes

Anastomotic leakage was defined as any defect at the anastomotic site confirmed on imaging procedures, examination under anaesthesia or by surgical re-intervention, (requiring radiologic placement of a pelvic drain, transanal lavage, endosponge placement or ileostomy creation).

Statistical Analysis

Continuous variables were presented as mean values with standard deviation (SD) or as median values with an interquartile range (IQR) according to the distribution. Discrete variables were presented as counts and percentages. Categorical data were compared between groups using the $\chi 2$ test, and continuous data were compared using the independent samples *t*-test or Mann Whitney U test according to distribution. Univariable and multivariable analysis were performed to identify predictive factors associated with anastomotic leakage, using the logistic regression method. The analyses were performed separately for patients that underwent a proctocolectomy and IPAA and for patients that underwent a completion proctectomy and IPAA. In univariable regression, variables with a two-sided p- value < 0.20 were considered for inclusion in multivariable analysis. Based on literature (as well as clinical practice) the factors Crohn's disease, hand-sewn anastomosis, laparoscopic approach and a defunctioning ileostomy were considered as clinically important factors, possibly influencing the outcome and were therefore also entered in the logistic regression.[18–20] With the use of a forward and backward selection regression method, factors were identified and included in our model. Variables with a p value ≥ 0.05 at each step of regression analysis were rejected. In multivariable analysis a p-value < 0.05 was considered significant, thus considered an independent risk factor. All statistical analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, Ill, USA).

RESULTS

A total of 640 patients were included in this study (339 AMC, 241 UZ Leuven, 60 Mayo). Three hundred sixty four (56.9%) patients were male with a median age of 38 years (IQR 29 – 48) at the time of pouch surgery. The preoperative diagnosis was UC in 564 (88.1%), indeterminate colitis in 58 (9.1%) and Crohn's Disease (CD) in 18 (2.8%) patients. A total of 96 (15.0%) patients in the study cohort had an anastomotic leak. There were no significant differences in leak rate between the institutes (AMC 14.7% versus UZ Leuven 14.1% versus Mayo 20.0%, p = 0.511). The median length of hospital stay was 11 days (IQR 8 – 17). The patient and surgical characteristics are shown in **table 1**.

Proctocolectomy and IPAA

In patients that underwent a proctocolectomy and IPAA, we found twelve variables that had a p value of less than 0.2 or were clinically relevant and were entered in the logistic regression: being overweight (BMI > 25), diabetes, ASA score (> 2), Crohn's disease, disease course (> 5 years), preoperative steroid treatment (≥ 20mg), anti-TNF treatment (< 3 months before IPAA), concurrent combination treatment of anti-TNF and steroids, handsewn anastomosis, laparoscopic approach, perioperative blood transfusion and defunctioning ileostomy. Multivariable regression analysis demonstrated long-term disease course (OR 2.19; 95%CI 1.15 – 4.17) and concurrent treatment of anti-TNF and steroids (OR 5.82; 95%CI 1.50 – 22.61) as independent risk factors for anastomotic leakage (**Table 2**).

Table 1. Demographic and surgical characteristics of all patients that underwent pouch surgery

	Total cohort (n = 640)	No anastomotic leakage (n = 544)	Anastomotic leakage (n = 96)	P value
Male (n, %)*	364 (56.9)	309 (56.8)	55 (57.3)	1.000
Age at surgery*†	38 (30 – 47)	38 (29 - 46)	39 (32 - 49)	0.485
Smoking	67 (10.9)	55 (10.5)	12 (13.2)	0.467
BMI†				0.027
< 18 (underweight)	43 (7.3)	37 (7.4)	6 (6.7)	
18 – 25 (normal)	371 (63.3)	326 (65.6)	45 (50.6)	
26 – 30 (overweight)	124 (21.2)	97 (19.5)	27 (30.3)	
> 30 (obese)	48 (8.2)	37 (7.4)	11 (12.4)	
Weight loss > 5% (n, %)	73 (11.5)	63 (11.6)	10 (10.4)	0.862
Previous abdominal surgery	124 (19.4)	105 (19.3)	19 (20.0)	0.888
Diabetes	24 (3.8)	18 (3.3)	6 (6.2)	0.154
CCI (> 3)	29 (4.5)	22 (4.1)	7 (7.3)	0.180
ASA score*				0.039
I	197 (32.8)	173 (34.1)	24 (26.1)	
II	315 (52.5)	268 (52.8)	47 (51.1)	
III	88 (14.7)	67 (13.2)	21 (22.8)	
Diagnosis*				0.668
Ulcerative colitis	564 (88.1)	480 (88.2)	84 (87.5)	
Indeterminate colitis	58 (9.1)	50 (9.2)	8 (8.3)	
Crohn's disease	18 (2.8)	14 (2.6)	4 (4.2)	
Disease course (years)†	5 (2 – 10)	4 (2 – 9)	8 (3 – 13)	0.001
Steroid therapy (≥20mg)	128 (21.3)	100 (19.6)	28 (30.8)	0.025
Anti-TNF ever	192 (31.3)	161 (31.0)	31 (32.6)	0.810
Anti-TNF (3 month preop)	51 (8.3)	39 (7.5)	12 (12.6)	0.106
Anti-TNF & steroids‡	18 (2.9)	11 (2.1)	7 (7.4)	0.012
Preop haemoglobin (< 5mmol/L)	43 (8.2)	35 (7.8)	8 (10.8)	0.364
Emergency surgery*	58 (9.2)	54 (10.2)	4 (4.2)	0.082
Stages*				0.130
1-stage	150 (23.4)	120 (22.1)	30 (31.2)	
2-stage	208 (32.5)	175 (32.2)	33 (34.4)	
Modified 2-stage	189 (29.1)	163 (30.0)	23 (24.0)	
3-stage	96 (15.0)	86 (15.8)	10 (10.4)	
Type pouch				0.172
J-Pouch	570 (91.3)	492 (90.9)	91 (95.8)	
Other (W,B,S)	54 (8.7)	49 (9.1)	4 (4.3)	
Anastomosis				0.339
Stapled	606 (96.8)	512 (96.4)	94 (98.9)	
Hand sewn	20 (3.2)	19 (3.6)	1 (1.1)	
Approach*				0.267
Laparoscopic	291 (45.5)	242 (44.6)	49 (51.0)	
Open	348 (54.4)	301 (55.4)	47 (49.0)	
Perioperative blood transfusion	76 (15.3)	61 (14.2)	15 (22.4)	0.100
Defunctioning ileostomy*	304 (47.5)	261 (48.0)	43 (44.8)	0.581
Length of stay after IPAA (days)*†	11 (8 – 14)	11 (8 – 13)	16 (10 – 23)	< 0.0001
Follow up (months)†	35 (10 – 88)	34 (10 – 88)	36 (9 – 89)	0.616

*Prospective data

† Data expressed as medians and IQR

‡ Concurrent treatment of anti-TNF and steroids (≥ 20mg) within 3 months before IPAA surgery

BMI: body mass index, CCI: Charlson's Comorbidity Index, ASA: American Society of Anaesthesiologists classification

Variables containing missing data: Smoking, 27 (4.2); BMI, 54 (8.4); Weight loss, 3 (0.5); Previous abdominal surgery, 1 (0.2); CCI, 1 (0.2); ASA score, 40 (6.3); Steroid therapy, 40 (6.3); Anti-TNF ever, 26 (4.1); Anti-TNF 3month preop, 26 (4.1); Anti-TNF & steroids, 29 (4.5); Preop haemoglobin, 116 (18.1); Emergency surgery 12, (1.9); Type pouch, 16 (2.5); Anastomosis, 14 (2.2); Approach, 1 (0.2); Perioperative blood transfusion 144 (22.5)

Table 2. Univariable and multivariable analysis of risk factors for anastomotic leakage in patients after proctocolectomy and IPAA

	Univariable analysis OR (95%CI)	P value	Multivariable analysis Adjusted OR (95%CI)	P value
Overweight (BMI > 25)	1.85 (1.03 – 3.32)	0.041	1.78 (0.93 – 3.39)	0.081
Diabetes	3.93 (1.03 – 15.08)	0.046		
CCI (> 3)	1.72 (0.65 – 4.56)	0.274		
ASA (> 2)	1.55 (0.81 – 2.97)	0.184	2.03 (0.90 – 4.56)	0.087
Crohn's disease*	2.39 (0.43 – 13.32)	0.322		
Disease course (> 5 year)	1.78 (1.02 – 3.10)	0.041	2.19 (1.15 – 4.17)	0.018
Steroid therapy (≥20mg)	1.51 (0.86 – 2.67)	0.153		
Anti-TNF (3 month preop)	1.89 (0.89 – 4.03)	0.099		
Anti-TNF & steroids	2.98 (1.04 – 8.53)	0.042	5.82 (1.50 – 22.61)	0.011
Emergency surgery	0.51 (0.06 – 4.09)	0.525		
J-pouch versus others	1.44 (0.41 – 4.99)	0.569		
Handsewn anastomosis*	2.19 (0.27 – 17.46)	0.458		
Laparoscopic approach*	1.24 (0.70 – 2.19)	0.454		
Perioperative blood transfusion	2.07 (0.96 – 4.45)	0.062		
Defunctioning ileostomy*	0.75 (0.44 – 1.30)	0.312	0.50 (0.25 – 1.00)	0.051

* Clinically relevant variables included based on previous literature

Completion proctectomy and IPAA

In patients that underwent completion proctectomy and IPAA, univariable analysis identified being overweight (BMI > 25), ASA score (> 2), disease course (> 5 years), concurrent combination treatment of anti-TNF and steroids and emergency surgery for logistic regression analysis. Subsequently, multivariable analysis demonstrated being overweight (OR 2.39; 95%CI 1.05 – 5.41) and high ASA score (OR 5.05; 95%CI 1.36 – 18.79) as independent risk factors (**Table 3**).

Table 3. Univariable and multivariable analysis of risk factors for anastomotic leakage in patients after completion proctectomy and IPAA

	Univariable analysis OR (95%CI)	P value	Multivariable analysis Adjusted OR (95%CI)	P value
Overweight (BMI > 25)	2.33 (1.08 – 5.02)	0.031	2.39 (1.05 – 5.41)	0.037
Diabetes	1.17 (0.25 – 5.44)	0.840		
CCI (> 3)	1.52 (0.17 – 13.41)	0.707		
ASA (> 2)	2.81 (0.94 – 8.38)	0.065	5.05 (1.36 – 18.79)	0.016
Crohn's disease*	1.54 (0.32 – 7.36)	0.587		
Disease course (> 5 year)	2.17 (1.04 – 4.54)	0.039	2.16 (0.96 – 4.86)	0.063
Steroid therapy (≥20mg)	2.22 (0.44 – 11.18)	0.335		
Anti-TNF (3 month preop)	0.71 (0.09 – 5.70)	0.744		
Anti-TNF & steroids	7.28 (0.44 – 119.30)	0.164	(-)	1.000‡
Emergency surgery	0.43 (0.13 – 1.48)	0.180		
J-pouch versus others	1.94 (0.44 – 8.59)	0.381		
Hand sewn anastomosis*	(-)†	0.603‡		
Laparoscopic approach*	0.90 (0.39 – 2.10)	0.809		
Perioperative blood transfusion	1.00 (0.28 – 3.56)	1.000		
Defunctioning ileostomy*	0.82 (0.38 – 1.81)	0.630	0.73 (0.29 – 1.87)	0.522

* Clinically relevant variables included based on previous literature
† Due to insufficient events (n = 0) in the handsewn group OR cannot be calculated
‡ Calculated by Fisher Exact Test

DISCUSSION

To enable a tailored surgical strategy (staged approach) in patients undergoing IPAA surgery, reliable identification of patients at high risk for anastomotic leakage is of utmost importance. Risk stratification will influence surgical decision-making and patient preparation. The focus of the current multicentre study was to identify pre- and peroperative predictive parameters associated with anastomotic leakage. Since we believe that there are two different patient categories with different risk profiles, that is primary proctocolectomy with IPAA patients and the completion proctectomy and IPAA patients, we analysed them separately. This study identified long-term disease course and the concurrent combination of steroid and anti-TNF treatment before IPAA surgery as independent risk factors for anastomotic leakage in IBD patients that underwent a proctocolectomy and IPAA. Being overweight and high ASA score were independent risk factors in patients that underwent a completion proctectomy and IPAA at a later stage. Interestingly, a defunctioning ileostomy did not show a reduced leak rate for both these procedure strategies, whereas a completion proctectomy and IPAA at a later stage reduced the leak rate by almost 50%.

Previous studies identifying parameters associated with an increased leak rate show comparable results for being overweight, ASA score and steroid treatment.[16,21,22] Other reported variables, such as low preoperative haemoglobin levels (< 10g/L), a CCI score of 3 or more and male sex, had no influence on the anastomotic leak rate in our study.[3,23] Although one study has shown that diabetes and Crohn's disease were strong predictors for pouch failure, no study has found any influence on the anastomotic leak rate.[18] In line with this, our study did not show an association between diabetes or Crohn's disease and anastomotic leakage, possibly due to the low incidence of these patients in our pouch cohort.

To our knowledge, the association between prolonged disease course and anastomotic leakage has not been evaluated before. Our study is the first to show a strong significant association between a disease course of more than 5 years and anastomotic leakage. Whether this increased risk is due to the influence of prolonged medication use or the chronic state of the disease that might have impaired anastomotic healing should be evaluated in future studies.

In literature, contradicting results have been reported regarding the association between anti-TNF and anastomotic leakage. A meta-analysis by Yang et al., initially showed a significant association between preoperative anti-TNF use and postoperative complications, but the results were disputed when more studies where added a few years later.[13,24] In a recent meta-analysis of 21 studies, an increased risk for postoperative infectious complications was found in Crohn's disease patients who underwent preoperative anti-TNF therapy.[25] A meta-analysis by Narula et al. showed somewhat similar results, with an increased risk for overall postoperative complications in IBD patients using preoperative anti-TNF treatment.[12] In our study, in univariable analysis, the association between anti-TNF treatment and anastomotic leakage almost reached significance in patients who had been treated within 12 weeks prior to IPAA surgery, and did not reveal a trend in patients using anti-TNF for more than 12. It therefore seems that the 12-week window proposed in other studies is an appropriate cut off point after which a lower risk for anastomotic leakage may be anticipated. This in line with the observation that anti TNF can be detected up to 12 weeks after last administration.[17]

Although previous studies have shown a significant association between high dose steroid treatment (≥ 20mg) and postoperative complications, studies evaluating the impact of concurrent anti-TNF and steroid therapy are lacking.[26,27] Therefore, our aim was to evaluate the additive effect of anti-TNF on steroids. One may argue that the need for concomitant medication, including steroids, is indicative for a more severe disease course. Thus, any potential adverse effect detected in these patients might be related to the fact that they were in a worse health state before IPAA surgery. However, the effect of anti-TNF in combination with steroids was demonstrated to be an independent risk factor in multivariable analysis, including both parameters separately, and remained a strong independent risk factor after adjustment for other predictive risk factors.

A defunctioning ileostomy is generally regarded as a protective measurement which reduces the risk of symptomatic anastomotic leakage by almost 50%, thereby minimizing the risk of septic complications.[20] Controversy exists in routine placement, based on increasing evidence about morbidity of ileostomy closure and prolonged hospitalization.[28] Morbidity rates have been reported to be up to 18% with small bowel obstruction being the most common complication after ileostomy reversal.[1,29] Interestingly, we did not find any differences in leak rate among patients with a defunctioning stoma when compared to non-deviated patients. However, when patients were in a better condition and weaned of medication the leakage rate decreased to approximately 10%. Considering the aforementioned morbidity after ileostomy reversal, a modified 2-stage procedure with subtotal colectomy and subsequent completion protectomy with IPAA formation (without an ileostomy) seems a preferable alternative strategy to diminish the risk for anastomotic leakage.

The main limitation of our study is mostly due to its retrospective nature. Although, all three hospitals prospectively maintain a procedural database, additional data was gathered retrospectively by chart review. Therefore, several variables that have been described in previous studies to have an effect on the anastomotic leak rate could not be evaluated in our cohort.[21,30] First, we were unable to include preoperative albumin levels and white blood cell counts, because these data were often unavailable. Second, we encountered a high percentage of missing data (> 30%) for intraoperative variables such as the number of firings of the stapling devices or anastomotic tension, prohibiting inclusion in our analysis. Prospective controlled studies are necessary to gather a complete set of variables in order to develop an accurate prediction tool.

Our study suggests that long-term disease course of more than 5 years and concurrent combination of anti-TNF and steroid treatment were independent preoperative risk factors for anastomotic leakage after proctocolectomy and IPAA. In contrast, being overweight (BMI > 25) and a high ASA score (> 2) remain significant risk factors after a modified 2-stage procedure. These readily available preoperative risk factors can be used in daily practice to guide clinical decision-making.

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CHAPTER 8

Defunctioning ileostomy is not associated with reduced leakage in proctocolectomy and ileal pouch anastomosis surgeries for IBD

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ABSTRACT

Background: Anastomotic leakage is a serious complication after restorative proctocolectomy with ileal pouch-anal anastomosis. Previous studies have shown significantly decreased leak rates in diverted patients with less severe clinical consequences. The aim of this study was to evaluate short and long term outcome of selective ileostomy formation in a multicentre cohort of patients undergoing pouch surgery.

Methods: In a retrospective study, 621 patients undergoing pouch surgery for inflammatory bowel disease (IBD) were identified from three large centres. Anastomotic leakage was defined as any leak confirmed by either contrast extravasation on imaging or during surgical re-intervention.

Results: In 305 patients (49.1%), primary defunctioning ileostomy was created during pouch surgery and 41 (6.6%) patients received a secondary ileostomy because of a leaking non-diverted pouch. Primary ileostomy formation was associated with male sex, weight loss, ASA > 2, steroid use, one-stage surgery, hand-sewn anastomosis and blood transfusion. Leak rates were comparable between diverted and non-diverted patients (16.7% vs 17.1%, p=0.92), which remained unchanged in subgroups with immunosuppressive medication. Having had an ileostomy was demonstrated to be an independent predictor of small bowel obstruction (OR 2.58, 95%CI 1.45 – 4.67) and pouch fistulas (OR 3.05, 95%CI 1.06 – 8.73). The 10-year pouch survival was comparable for patients with and without ileostomy (89% versus 88%, P=0.718).

Conclusions: Leakage rates of diverted and non-diverted pouches in IBD patients were similar and relatively high. Defunctioning was independently associated with long-term complications. A staged approach without defunctioning might be the best strategy.

INTRODUCTION

Anastomotic leakage after proctocolectomy with ileal anal pouch anastomosis (IPAA) is a serious complication with a reported incidence that varies between 10-15%.[1,2] Nowadays, an IPAA is indicated for a considerable large proportion of therapy refractory ulcerative colitis (UC) patients (20-30%), all familial adenomatous polyposis (FAP) patients and selected cases of Crohn's disease.[3] UC patients have a higher risk for anastomotic leakage due to the inflammatory state of the colon. Previous studies have shown various risk factors such as steroids and biologicals that increase the risk for anastomotic leakage.[4,5] Furthermore, a trend has been seen towards more extensive medical treatment in inflammatory bowel disease (IBD) patients, leaving refractory patients in a worse condition when it comes to surgery.

Widespread attention has been paid towards new strategies in order to prevent or diminish the consequences of anastomotic leakage. In most practices, surgeons perform a multistage IPAA, involving the creation of a temporary defunctioning ileostomy as a protective measure for postoperative anastomotic leakage.[6] It has been suggested that a defunctioning ileostomy avoids pressure on the fresh suture line, thus reducing the risk for leakage. A previous meta-analysis of 1483 patients confirms this hypothesis by showing a two-fold higher risk for leakage in non-deviated patients when compared to patients with a protective ileostomy. In contrast, high morbidity rates have been reported after ileostomy reversal while prevention of leakage is not guaranteed. A large study of 1,504 patients reported a complication rate of 11.4% after ileostomy reversal.[7]

Currently, no consensus has been reached regarding routine defunctioning ileostomy in ileoanal pouch surgery. The objective of this multicentre cohort study was to evaluate short and long term outcome of selective ileostomy formation in a multicentre cohort of patients undergoing IPAA for IBD.

METHODS

Between September 1990 and May 2014, consecutive IBD patients who underwent IPAA and were followed for at least one year were identified from prospectively maintained procedural databases of the Academic Medical Center (AMC), Amsterdam, The Netherlands and University Hospitals Leuven (UZ Leuven), Belgium. From the Mayo clinic in Scottsdale Arizona USA, patients undergoing IPAA between March 2009 and July 2012 were included. All three hospitals are tertiary Referral Centres with an annual volume of approximately 20-35 IPAA procedures. Approval by the medical ethical boards was not required due to the observational design of the study.

Within all three institutes, experienced colorectal surgeons or supervised colorectal fellows performed IPAA procedures. Patients were treated in an elective setting by a 1-stage procedure being a proctocolectomy with IPAA creation, or a 2-stage procedure, which is a proctocolectomy with IPAA creation and defunctioning ileostomy, followed by reversal at the second stage. In a more acute setting, patients were treated by a 3-stage procedure, being a subtotal colectomy with end-ileostomy, followed by completion proctectomy with IPAA and defunctioning ileostomy, and ileostomy reversal at the third stage. In case a defunctioning ileostomy was omitted from the 3-stage procedure, the procedure was called a 'modified' 2-stage. At the AMC and UZ Leuven, defunctioning ileostomy was created selectively at the surgeon's discretion (e.g. medication, severe proctitis, mucosectomy with a hand sewn anastomosis, an incomplete 'doughnut' after stapling, or an anastomosis with high tension). At the Mayo clinic, all patients were defunctioned. Pouchography/endoscopy was performed before ileostomy reversal in all 3 hospitals.

Demographic (sex, age at surgery, smoking habits, BMI), preoperative (weight loss, comorbidities, the American Society of Anaesthesiologists score (ASA score), diagnosis, medications use) and surgical variables (emergent surgery, type of resection/anastomosis/procedure, perioperative blood transfusion, length of stay, follow up) were retrospectively extracted from patients' charts. Short-term and long-term outcomes were also recorded. Overweight was defined as the body mass index (BMI) > 25 kg/m^2. The Charlson comorbidity index (CCI) scores were calculated for each individual patient by weighing their comorbidities and adding 1 point for each decade over the age of 40 years. The CCI was dichotomized with a cut-off point of 3, and the ASA score with a cut-off point of 2. Preoperative steroid and anti-TNF use were defined as patients using medication up to 12 weeks prior to IPAA. Perioperative blood transfusion is defined as all in hospital transfusions that took place before or during surgery.

Outcomes

The primary, short-term outcome measure was anastomotic leakage. Anastomotic leakage was defined as any defect at the anastomotic site confirmed by imaging or during surgical re-intervention and was categorized according to the impact on clinical management (A, B, C). Grade A leaks had minimal to no clinical impact on the patients postoperative course, requiring antibiotics at the most. Grade B leaks required active intervention such as radiologic placement of a pelvic drain or transanal lavage. Grade C leaks required a re-operation mostly because the patient was not defunctioned. A secondary ileostomy was fashioned for clinically symptomatic leakage if antibiotic and/or drainage therapy alone was considered not to be sufficient.

The secondary, long-term outcome measures were small bowel obstruction (SBO), anastomotic stricture, fistulas related to the pouch, Crohn's of the pouch and chronic pouchitis. SBO was defined as such if adhesions causing the symptoms were found during surgery, or if confirmed by imaging. Anastomotic stricture was defined as narrowing at the IPAA requiring dilatation. Only fistulas that were related to the pouch where considered for evaluation as a long-term outcome. The strictures and fistulas that were due to Crohn's disease were considered as Crohn's of the pouch. We considered pouchitis as chronic, when a patient depended on antibiotics or required escalation to treatment with 5-aminosalicylates, corticosteroids, immunomodulatory therapy, or anti-TNF agents. Pouch excision, permanent pouch diversion, or the need for an abdomino-perianal pouch salvage procedure (redo-IPAA or neo-IPAA following excision) were all considered as pouch failure.

Statistical Analysis

Continuous variables were presented as mean values with a standard deviation (SD) or as median values with an interquartile range (IQR) according to the distribution. Discrete variables were presented as counts and percentages. Categorical data were compared between groups using the $\chi 2$ test, and continuous data were compared using the independent samples *t*-test or Man Whitney U test. A subgroup analysis was performed comparing the anastomotic leak rate between the primary ileostomy group and non-ileostomy group in patients treated with steroids, anti-TNF or a combination of steroids and anti-TNF, separately. The Kaplan-Meier method was used to compare the cumulative pouch failure rates between the primary ileostomy and non-ileostomy group, using the log rank test. Univariable and multivariable logistic regression analysis was performed to evaluate the independent association between ileostomy formation and long-term complications. In univariable regression, all baseline variables with a two-sided p- value of less than 0.2 were considered for inclusion in multivariable analysis. As advances in surgical techniques over time may have been a confounding factor, we added a variable comparing the defunctioning ileostomy rates before and after the year 2000 in multivariable analysis. A total of 5 variables were included in the multivariable model for SBO, 5 variables for the multivariable model for strictures, 7 variables for the pouch fistulas model and 6 variables for the chronic pouchitis model. Variables with a two-sided p- value of less than 0.05 were considered significant. All statistical analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, Ill, USA).

RESULTS

A total of 621 patients were included in this study (314 AMC, 247 UZ Leuven, 60 Mayo). Three hundred and fifty one (56.5%) patients were male, and the median age was 38 years (6 – 77) at the time of IPAA. In 305 patients (49.1%) a primary defunctioning ileostomy was fashioned. In 211 (69.2%) of those patients, a 2-stage procedure was performed and in 94 (30.8%) a 3-stage procedure. In the 316 patients without a primary ileostomy, a modified 2-stage procedure was performed in 165 (52.2%) patients and a one-stage procedure in the remaining 151 (47.8%) patients (**Figure 1**). A secondary ileostomy for leakage was constructed in 26 (7.2%) patients after a one-stage procedure, and 15 (5.8%) patients after a modified 2-stage procedure. The median time to ileostomy reversal was 3 months (IQR, 2-3), which was comparable between all three hospitals. The median follow up time was 39 months (IQR, 11–94) (**Table 1**).

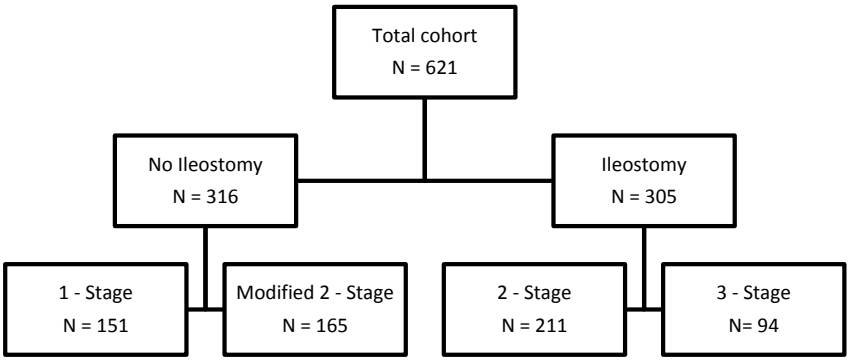

Figure 1. Flow diagram of the pouch procedure stages in patients with and without defunctioning ileostomy

Factors associated with ileostomy formation

In univariable analysis, patients in whom a primary defunctioning ileostomy was fashioned were more often males, showed more weight loss, and had a high ASA score (62.4%vs 51.6%, p = 0.010; 14.5% vs 8.5%, p=0.029 and 29.0 % vs 3.2%, p=<0.001; respectively) when compared to the non-ileostomy group. Other demographic data were similar between both groups. In the ileostomy group, significantly more patients were treated with steroids (29.5% vs 12.1%, p<0.001) before IPAA when compared to patients without ileostomy. Patients who underwent a subtotal colectomy followed by a completion proctectomy and IPAA, where less likely to have an ileostomy when compared to patients who underwent a proctocolectomy with IPAA (31.8% vs 52.7%, p<0.001). Patients in whom a defunctioning ileostomy was fashioned, more often had a hand sewn anastomosis (6.6% vs 1.0%, p=0.001) and perioperative blood transfusions (21.2% vs 13.2%, p=0.014) when compared to patients without ileostomy (**Table 1**).

Table 1. Baseline characteristics of patients with and without a defunctioning ileostomy

	Total (n=621)	Ileostomy (n= 305)	No ileostomy (n= 316)	P value
Male (n, %)	351 (56.5)	188 (61.6)	163 (51.6)	0.012
Age at surgery†	38 (30 – 47)	39 (30 – 47)	38 (29 – 47)	0.335
Smoking	65 (11.0)	33 (11.1)	32 (10.9)	1.000
Overweight (BMI > 25)	144 (25.5)	75 (26.5)	69 (24.5)	0.630
Weightloss > 5% (n, %)	71 (11.5)	44 (14.6)	27 (8.5)	0.023
Previous abdominal surgery	119 (19.2)	57 (18.8)	62 (19.6)	0.839
CCI score (> 3)	39 (6.3)	15 (4.9)	24 (7.6)	0.188
ASA score (≥ 3)	90 (15.5)	80 (30.2)	10 (3.2)	<0.001
Diagnosis				0.073
Ulcerative colitis	545 (87.8)	277 (90.8)	268 (84.8)	
Indeterminate colitis	59 (9.5)	22 (7.2)	37 (11.7)	
Crohn's disease	17 (2.7)	6 (2.0)	11 (3.5)	
Steroids (> 20mg)	129 (22.3)	93 (33.0)	36 (12.1)	<0.001
Anti-TNF (3 month preop)	51 (8.5)	37 (12.6)	14 (4.6)	<0.001
Emergent surgery	49 (8.1)	22 (7.4)	27 (8.8)	0.554
Type of resection				<0.001
Proctocolectomy*	362 (58.3)	211 (69.2)	151 (47.8)	
Completion proctectomy**	259 (41.7)	94 (30.8)	165 (52.2)	
Hand sewn anastomosis	20 (3.3)	17 (5.6)	3 (1)	0.001
Open procedure	332 (53.5)	169 (55.4)	163 (51.8)	0.376
Perioperative blood transfusion	78 (16.4)	38 (22.1)	40 (13.2)	0.014
Secondary ileostomy	45 (7.2)	0	45 (14.2)	-
Postoperative length of stay after IPAA†	11 (8 – 14)	11 (8 – 14)	11 (9 – 15)	0.003
Follow up (months)†	39 (11 – 94)	50 (14 – 132)	35 (10 – 75)	<0.001

† Data expressed as medians and IQR
* One-stage versus 2-stage procedure
** Modified 2-stage versus 3-stage procedure
BMI: body mass index, CCI: Charlson's Comorbidity Index, ASA: American Society of Anesthesiologists class
Variables containing missing data: Smoking, n=29 (4.7); Overweight, 56 (9.0); Weightloss, n=3 (0.5%); Previous abdominal surgery, n=1 (0.2); CCI score, n=1 (0.2); ASA score, n=40 (6.4); Steroids, n=42 (6.8); Anti-TNF, n=22 (3.5); Hand sewn anastomosis, n=13 (2.1); Open procedure, n=1 (0.2); Perioperative blood transfusion, n=145 (23.3)

Anastomotic leak rate in patients with and without ileostomy

A total of 105 patients (16.9%) had a leak in the study cohort, of whom 15 (14.3%) were grade A, 44 (41.9%) grade B and 46 (43.8%) grade C. The leak rate was comparable between all three hospitals (AMC 16.2%, UZ Leuven 17.0%, Mayo 20.0%, p = 0.759). A comparable overall leak rate was found in the ileostomy group when compared to patients without ileostomy (16.7% vs 17.1%, p = 0.921). As expected, significantly fewer patients required re-

operation in the ileostomy group (10.4% vs 4.3%, p = 0.003), because the leaking anastomosis was already defunctioned. There was no difference when combining the clinically relevant leaks (grade B and C; 13.8% vs 15.2%p = 0.649) (**Table 2**).

Subgroup analysis of patient with preoperative treatment

In all patients treated with steroid therapy within 3 months before surgery, comparable anastomotic leak rates were found among patients with a defunctioning ileostomy and those without a stoma (20.4% vs 25.0%, p=0.639). A similar leak rate for diverted and non-diverted IPAA was shown for patients who received preoperative anti-TNF therapy within 3 months before surgery (24.5% vs 21.4%, p=1.000). In patients that were concurrently treated with steroids and anti-TNF, similar high leak rates (40.0% vs 33.3%, p=1.000) were found in patients with a protective ileostomy and those without ileostomy, respectively (**Figure 2**).

Table 2. Short term and long term outcomes after IPAA in patient with and without a primary ileostomy

	Ileostomy (n= 305)	**No ileostomy (n= 316)**	**P value**
Short term complication			
Overall leak rate	51 (16.7)	54 (17.1)	0.915
Anastomotic leakage by grade			0.001
Grade A	9 (3.0)	6 (1.9)	
Grade B	29 (9.5)	15 (4.7)	
Grade C	13 (4.3)	33 (10.4)	
Clinically relevant leak (grade B&C)	42 (13.8)	48 (15.2)	0.649
Long term complications			
Small bowel obstruction	57 (18.9)	32 (10.3)	0.003
Stricture	32 (10.6)	15 (4.9)	0.009
Abdominal hernia	5 (1.7)	3 (1.0)	0.501
Fistulas related to the pouch	35 (11.6)	9 (2.9)	<0.001
Crohn's of the pouch	6 (2.0)	15 (4.7)	0.074
Chronic pouchitis	42 (15.4)	26 (9.1)	0.027
Pouch failure	22 (7.3)	20 (6.3)	0.750

Variables containing missing data: Small bowel obstruction, n=9 (1.4); Stricture, n=9 (1.4); Abdominal hernia, n=9 (1.4); Fistulas related to the pouch, n=9 (1.4); Chronic pouchitis, n=64 (10.3); Pouch failure, n=2 (0.3)

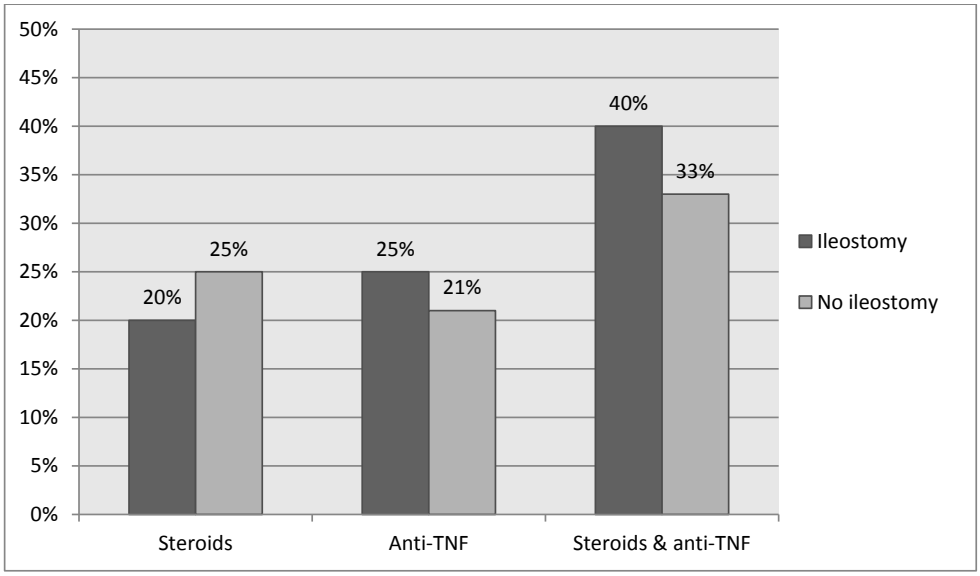

Figure 2. Subgroup analysis for steroids, anti-TNF and a combinations of steroids and anti-TNF, comparing the anastomotic leak rate between the ileostomy group and non-ileostomy group (all associations P > 0.05)

Long-term postoperative complications

The long term follow up of patients with and without defunctioning ileostomy showed similar outcomes considering abdominal hernias and pouch failure (p> 0.05). Patients, in whom a defunctioning ileostomy was fashioned, had a higher risk of SBO (18.9%, versus 10.3% p=0.003), strictures (10.6%, versus 4.9% p=0.009), fistulas (11.6% versus2.9%, p<0.001) and chronic pouchitis (15.4%, versus 9.1% p=0.027) (Table 2). The odds ratios for SBO and fistulas remained significant when controlling for confounding variables (**Table 3**). The 10-year cumulative pouch survival was comparable for patients with and without ileostomy (89% versus 88%, P=0.718) (**Figure 3**).

Table 3. Multivariable analysis: the independent association between ileostomy formation and long-term complications

	Small bowel obstruction OR (95%CI)	**Strictures** OR (95%CI)	**Fistulas** OR (95%CI)	**Chronic pouchitis** OR (95%CI)
Primary ileostomy	2.58 (1.45 – 4.67)*	1.59 (0.78 – 3.24)*	3.05 (1.06 – 8.73)*	1.57 (0.82 – 3.02)*
Sex	0.47 (0.28 – 0.78)	-	-	-
Age at surgery	-	-	-	-
Smoking	-	-	-	-
Overweight (BMI > 25)	0.66 (0.35 – 1.25)	0.21 (0.06 -0.69)	-	0.63 (0.31 – 1.28)
Weightloss > 5%	-	1.71 (0.78 – 3.73)	-	-
CCI score (> 3)	-	-	-	-
ASA score (≥ 3)	-	-	6.50 (2.13 – 19.79)	0.82 (0.37 – 1.80)
IC & CD	2.89 (1.45 – 5.76)	0.18 (0.02 – 1.36)	-	2.59 (1.28 – 5.22)
Steroids (> 20mg)	-	-	1.10 (0.37 – 3.31)	1.25 (0.65 – 2.38)
Anti-TNF	1.18 (0.49 – 2.84)	-	-	-
Emergent surgery	-	-	-	-
Proctocolectomy	-	-	0.45 (0.14 – 1.47)	-
Hand sewn anastomosis	2.09 (0.71 – 6.13)	1.16 (0.25 – 5.51)	2.18 (0.40 – 11.92)	-
Open procedure	-	-	1.65 (0.60 – 4.56)	-
Perioperative blood transfusion	-	-	1.91 (0.66 – 5.49)	-
Secondary ileostomy	-	-	-	1.26 (0.41 – 3.64)
Pouch after year 2000	-	0.55 (0.28 – 1.10)	4.83 (0.96 – 23.89)	0.50 (0.26 – 0.94)

*Adjusted for variables with a p value of < 0.2 in univariable analysis.
Variables with a p value of > 0.2 in univariable analysis are indicated by a (-) and excluded from multivariable analysis
BMI: body mass index, CCI: Charlson's Comorbidity Index, ASA: American Society of Anesthesiologists class, IC: Indeterminate colitis, CD: Crohn's disease

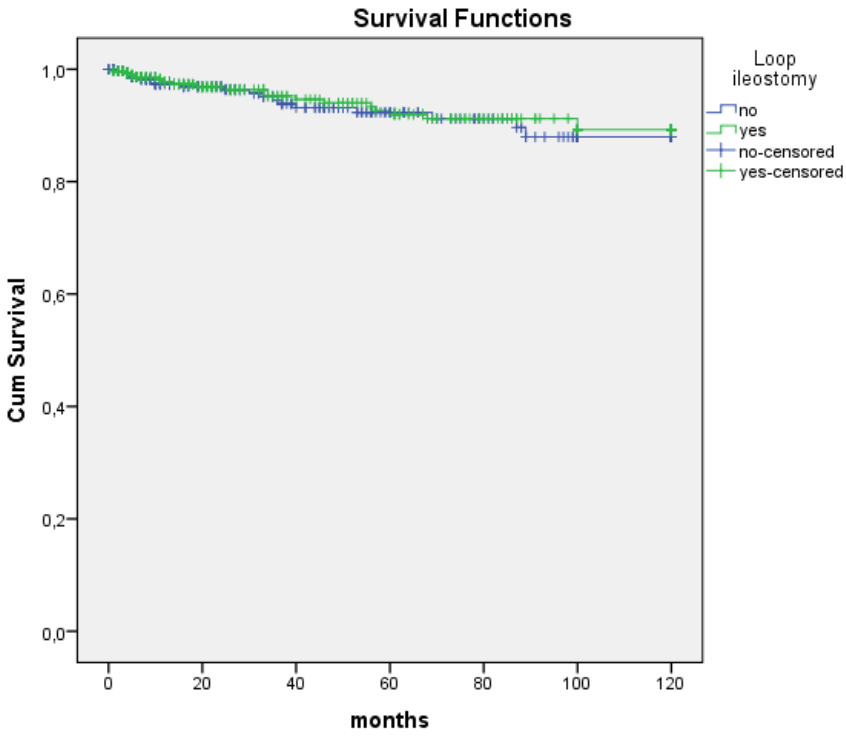

Figure 3. Kaplan-Meier pouch failure curves for patients with and without a primary ileostomy

DISCUSSION

The present study shows that the decision to defunction IPAA is related to gender, ASA-score, immunomodulatory treatment, one-stage proctocolectomy, a hand-sewn anastomosis and blood transfusion. Anastomotic leakage was high in all patients, even if the IPAA was defunctioned. Particularly the patients having steroids and anti-TNF had high leak rates which did not change by defunctioning the IPAA. Furthermore, primary ileostomy showed to be independently associated with long-term morbidity such as SBO, strictures and fistulas while no beneficial effect on the cumulative pouch failure rate were found.

The leak rate in our study was higher than previously shown which can be explained by the fact that we only included IBD patients.[1,2] It is well known that polyposis coli patients have lower leak rates than UC patients for obvious reasons eg. patients are younger, not diseased and do not take immunosuppressives. Another explanation might be the differences in definition of anastomotic leakage since no clear consensus has been reached so far.[8] Our numbers may also be explanation by the more precise definition and reporting of anastomotic leakage in our group. Also grade A leak rates were included.

There are a number of studies claiming benefit for primary defunctioning ileostomy in IPAA procedure.[8, 12] A previous study of 122 UC patients showed significantly lower pouch related septic complications when a defunctioning ileostomy was fashioned, resulting in a significantly lower second relaparotomy rate for the management of complications (4.5% vs. 30.3%, p<0.001).[11] In the current study, we found a high overall leak rate, which was comparable between patients with and without ileostomy. Considering only grade C leaks, differences between the ileostomy and non-ileostomy group might be overestimated. Operative re-intervention mainly consists of fashioning a defunctioning ileostomy as a consequence of deciding not to do so primarily. But this does not necessarily imply that those patients have more severe leakage. We therefore evaluated grade B and C leaks together and found comparable leakage rates for both groups. Still, one may argue that the anastomotic leak rate would have been even higher if the ileostomy was omitted in high-risk patients, supporting previous positive findings.

Patients who had been preselected for ileostomy due to steroids or anti-TNF usage remained to be in high risk for anastomotic leakage, especially when treated with both therapies concurrently. It is difficult to speculate how high the leak rates would have actually been in case no pre-selection of high-risk patients would have taken place. Still, we believe that the anastomotic leak rate is unacceptably high in patients who have been treated with steroids or anti-TNF, despite a protective ileostomy. Known risk factors that may increase the risk for anastomotic leakage include male sex, high ASA score, a high CCI score (>3), low preoperative haemoglobin levels (< 10g/L), preoperative albumin level lower than 3.5 g/dL, steroid treatment and biologicals.[12–17] Since leakage may not be prevented in high risk patients, a subtotal colectomy with end ileostomy and thereby postponing the IPAA procedure may be a better strategy instead of restoring continuity with a temporary defunctioning ileostomy.

Looking at the long-term outcomes, we found high complication rates associated with stoma closure, which were in line with previous studies. A systematic review of in total 48 studies (28 IBD related papers containing 3,277 patients), evaluated the morbidity and mortality following closure of loop ileostomy and reported an overall morbidity rate of approximately 17.3%. In 7.2% of the patients, SBO occurred after ileostomy closure of which one third requiring re-laparotomy and 1.3% suffered from a postoperative enterocutaneous fistula.[18] These rates were rather low compared to more recent studies, which may be explained by the fact that varying definitions were used and not all included papers reported the appropriate outcome measures. A recent study by the Cleveland Clinics showed a SBO rate of 17.9% of which almost 40% needed an intervention.[19] In their institution, it is standard practice to use a defunctioning ileostomy and only a few will not have a stoma under strict criteria. In the same study, a pouch stricture rate of 11.2% was shown, which was comparable to our results. A study by Lewis et al. showed a stricture rate of as high

as 34%, which was significantly associated with previous defunctioning ileostomy.[20] In our study the stricture rate was higher in the ileostomy group, but was no longer significant after controlling for confounding variables.

Other studies have shown that up to 60% of patients may suffer from recurring pouchitis and in only 5-10% to become chronic.[21] The aetiology of pouchitis is unclear but it is noteworthy that it is rarely seen in FAP patients in contrast to UC. In the current study, primary ileostomy was not associated to chronic pouchitis. A secondary ileostomy was independently associated to chronic pouchitis, which is most likely a reflection of the underlying anastomotic leakage and the severity of the disease. It is known that patients with more severe colitis preoperatively will have a higher chance of developing pouchitis. Pelvic sepsis is one of the main causes of pouch failure. Interestingly, Tulchinsky et al. demonstrated that a defunctioning ileostomy is associated with a lower rate of pouch failure, suggesting a beneficial effect in the treatment of pelvic sepsis.[22] In contrast, our study showed a comparable cumulative pouch failure rate between patients with and without ileostomy over a period of 10 years.

Considerable thought should be given before deciding on primary defunctioning an IPAA. The uncertainty about the advantages of a primary defunctioning ileostomy and the suggested association with long-term complications demand a different approach. Using close observation with CRP and CT with anal contrast at even the lowest level of suspicion of anastomotic leakage enables early re-intervention with secondary defunctioning ileostomy. The risk of chronic pelvic sepsis and pouch failure may be overcome with endosponge treatment followed by early transanal reconstruction of the anastomosis.[23]

The strength of this multicentre study is the international collaboration and its large sample size and long follow up. Nevertheless, some limitations should be evaluated critically due to the retrospective nature.

These results imply that in daily clinical practice surgeons perform a defunctioning ileostomy in the more fragile and disease affected patients. Considering the inefficacy of preventing anastomotic leakage and the long term sequelae of the defunctioning ileostomy, and the harm to the patients that did not need an ileostomy, a modified two stage procedure seems more appropriate avoiding a more risky one stage procedure in the presence of concomitant use of steroids and/or anti TNF. Colectomy with ileostomy first followed by completion proctectomy without an ileostomy will reduce the leak rate and unnecessary stomas in the majority of the patients improving short term and long-term outcomes.

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CHAPTER 9

External validation of a prognostic model of preoperative risk factors of pouch failure

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ABSTRACT

Aim: The Cleveland Clinic has proposed a prognostic model of preoperative risk factors of pouch failure. The model incorporates four predictive variables for pouch failure: completion proctectomy, handsewn anastomosis, diabetes mellitus and Crohn's disease. The aim of the present study is to perform an external validation of this model in a new cohort of patients having had an ileal pouch-anal anastomosis.

Method: Validation was performed in a multicenter cohort of 747 consecutive patients who had an ileal pouch-anal anastomosis between 1990 and 2015 in three tertiary care facilities, using a Kaplan-Meier survival analysis and Cox regression analysis. The performance of the model was expressed using the Harrell's concordance error rate. The primary outcome measure was pouch survival.

Results: In the study period, 45 patients (6.0%) had pouch failure, with a median time to pouch failure of 31 months [IQR 9 - 82]. Multivariable analysis showed hand sewn anastomosis to be the only significant independent predictor. Harrell's concordance error rate was 0.42, indicating poor performance. Anastomotic leakage and Crohn's disease of the pouch were strong postoperative predictors for pouch failure and showed a significant difference in pouch survival after 10 years ($p<0.001$).

Conclusion: The poor performance of the Cleveland Clinic prognostic model does not make it suitable for application in daily clinical practice. Handsewn anastomosis was associated with pouch failure in our cohort with relatively few events. A prediction model for anastomotic leakage or Crohn's of the pouch may be a better solution since these variables are strongly associated to pouch failure.

INTRODUCTION

Pouch failure is a feared complication after ileal pouch-anal anastomosis (IPAA). Reported incidence of pouch failure varies, but in large series with an extensive long-term follow-up, up to 10% failure rates have been reported.(1,2) Main causes of pouch failure are fistulizing disease, unrecognized Crohn's disease or chronic pelvic sepsis.(3) Patients often have longstanding complaints before definitive pouch deviation or excision is performed. Thus, if it were possible to determine whether certain patient characteristics or surgical characteristics lead to an increased risk of pouch failure, this could influence perioperative decision-making.

Recently a prognostic model has been proposed by the Cleveland Clinic.(1) In a series of 3754 patients who had an IPAA between 1983 and 2008 for any indication, a model using the novel random survival forest technique was computed to predict pouch survival. Internal validation showed a Harrell's concordance error rate of 0.32 (with a value of 0 denoting perfect accuracy).(1,4,5) The most informative variables (completion proctectomy, handsewn anastomosis, diagnosis of diabetes, diagnosis of Crohn's disease and age at surgery) were selected to subsequently generate a multivariable Cox proportional hazards model in which 4 variables were found to be independent predictors for pouch failure.

An important part of developing a prognostic model is external validation, in which the performance of the model is studied in a different population (validation set) to determine its generalizability. Such a validation set should be similar to the original cohort (derivation set) with regards to the index disease, or in this case, index operation, but it should differ for other cohort variables, e.g. timeframe or geography.(6,7)

The aim of this present study is to perform an external validation of the proposed prognostic model of preoperative risk factors of pouch failure in a multicenter cohort of patients with an IPAA.

METHODS

Patients and Data Collection

Patients were identified from a prospectively maintained database from three tertiary referral centers with dedicated IBD surgeons; Academic Medical Center (AMC) in Amsterdam, the Netherlands, the University Hospitals Leuven (UZ Leuven), Belgium and the Mayo Clinic (Mayo) in Scottsdale, Arizona USA. In the AMC and UZ Leuven, all patients having had an IPAA between September 1990 and February 2015 and in the Mayo all patients having had an IPAA between March 2009 and July 2012 were included. Subsequently, a retrospective chart review was performed of the identified patients to extract all clinical variables that

were used to create the Cleveland Clinic prognostic model, which included: sex, age, comorbidities, diagnosis, perioperative use of systemic steroids, the type of ileal anal pouch created, the type of anastomosis, total restorative proctocolectomy or completion restorative proctocolectomy, laparoscopic or open approach, and creation of a diverting ileostomy. In addition, we abstracted postoperative complications that may have occurred before pouch failure such as anastomotic leakage, small bowel obstruction (SBO), strictures, fistulas not related to Crohn's disease and Crohn's disease of the pouch.

Outcomes and definitions

Pouch failure was defined as either pouch excision, permanent pouch diversion, or the need for an abdominoperineal pouch salvage procedure (redo-IPAA or neo-IPAA following excision), similar to the Cleveland Clinic model.[1] Obesity was defined as such when the body mass index (BMI) was 30 kg/m^2 or higher. In the Cleveland Clinic prognostic model the variable 'diagnosis' was based on the postoperative diagnosis made by the pathologist. (1) This was done because in several cases there was no definitive preoperative diagnosis. Perioperative steroid use was defined as such when patients were still using systemic steroids at the day of the pouch procedure. Anastomotic leakage was defined as any defect at the anastomotic site confirmed on imaging procedures or during surgical re-intervention. Small bowel obstruction (SBO) was defined as such if adhesions causing the symptoms were found during surgery, or if confirmed by imaging. Stricture was defined as when narrowing at the IPAA required dilatation.

IPAA procedure

In 91% of the patients a J pouch was created. In the Cleveland Clinic series an S-pouch was created in 10% of the patients. In the Academic Medical Center, a B-pouch was created in a minority of patients (8.7%). The B-pouch is a modification of the J-pouch; the efferent loop of the J is anastomosed with the afferent loop creating a B shaped reservoir. Hand-assisted laparoscopic IPAA was carried out via a Pfannenstiel incision. At the AMC and UZ Leuven, a diverting ileostomy was only created on indication. Important indications were: steroids or biologicals, severe proctitis, mucosectomy with a handsewn anastomosis, an incomplete 'doughnut' after stapling or an anastomosis with high tension.

Statistical Analysis

Continuous variables were presented as mean values with a standard deviation (SD) or as median values with an interquartile range (IQR) according to the distribution. Discrete variables were presented as counts and percentages. No imputation was performed for missing data. For the purpose of the Kaplan-Meier and the Cox proportional hazard analyses a 'time to pouch failure' variable was created. Time to pouch failure was defined as the time

in months between the initial IPAA procedure and the date of pouch failure. Patients were censored if at the end of the follow-up period no pouch failure had occurred. Performance of the proposed risk factors was tested using Kaplan-Meier survival analysis with log-rank tests and Cox regression analysis. Predictive discrimination of the model was tested using Harrell's concordance error rate and corrected for over-optimism. The error rate is calculated as 1-C, in which C is the concordance index. A value of 0 is perfect and a value of 0.5 is no better than random guessing.(1,5) Exploratory analyses were performed to develop a pouch failure model within our own cohort with a follow up period of 120 months. The effect of different postoperative complications on pouch failure was analyzed using the Kaplan Meier analysis. All cases in which anastomotic leakage occurred or were diagnosed with Crohn's disease of the pouch were excluded from the analysis due to the strong correlation with pouch failure. Subsequently, independent risk factors for pouch failure were identified by multivariable Cox regression where all preoperative, surgical and postoperative variables with a two-sided p value of less than 0.02 were entered initially. All tests were two-sided and p values <0.05 were considered to be significant. Analysis was performed using IBM SPSS for Windows version 22 (SPSS Inc., Chicago, IL, USA) and R statistical software version 2.15.2 (The R Foundation for Statistical Computing) with the 'rms' and 'Hmisc' packages.

RESULTS

Cohort characteristics

In the study period, a total of 747 patients underwent an IPAA. All IPAA procedures were carried out by experienced colorectal surgeons or supervised colorectal fellows. Baseline characteristics of these patients are shown in **Table 1**. Median duration of follow-up was 33 months [IQR 9 - 85]. Overall, 45 patients (6.0%) had pouch failure, with a median time to pouch failure of 31 months [IQR 9 - 82]. **Table 2** shows the causes of pouch failure. Active Crohn's disease in the pouch was the most frequent cause of pouch failure.

Table 1. Patient characteristics of derivation set (n=3754) and validation set (n=747)

	Derivation set Cleveland Clinic 1983 - 2008 *n* = 3754	**Validation set Multicenter cohort**
Female	1656 (44.1)	327 (43.8)
Age at surgery (SD)	38.2 ± 13.3	39.1 ± 13.1
Age at diagnosis (SD)	29.4 ± 12.5	31.7 ± 12.4
Patients with comorbid conditions		
Pulmonary	291 (7.8)	26 (3.5)
Cardiovascular	243 (6.5)	32 (4.3)
Hypertension	366 (9.7)	39 (5.2)
Liver	169 (4.5)	49 (6.6)
Diabetes Mellitus	148 (3.9)	27 (3.6)
Renal	38 (1)	15 (2.0)
Obesity	361 (9.6)	57 (7.6)
Hypercoagulation	155 (4.1)	19 (2.5)
Diagnosis (postoperative histopathology)		
Ulcerative Colitis	2962 (81.1)	501 (67.1)
Indeterminate	63 (1.7)	46 (6.2)
Crohn's disease	150 (4.1)	35 (4.7)
Familial Adenomatous Polyposis	223 (6.1)	91 (12.2)
Cancer (& dysplasia)	97 (2.7)	74 (9.9)
Unknown/ other	158 (4.3)	0 (-)
Steroid therapy (perioperative)	1603 (45.6)	254 (34.0)
Length of stay *	7 [2 - 59]	11 (8 – 14)
Pouch type		
J-Pouch	3369 (89.7]	676 (90.5)
S-pouch	385 (10.3)	4 (0.5)
B-Pouch	0 (-)	65 (8.7)
Unknown/ other	158 (4.3)	2 (0.3)
Anastomosis		
Stapled	3233 (86.1)	674 (90.2)
Handsewn	521 (13.9)	39 (5.2)
Type of resection		
Total proctocolectomy	2238 (60.3)	435 (58.2)
Completion proctectomy	1475 (39.7)	312 (41.8)
Approach		
Laparoscopic	240 (6.4)	351 (47.0)
Open	3514 (93.6)	396 (56.9)
Defunctioning ileostomy	3273 (87.2)	322 (43.1)
Follow-up in months (median, IQR)	84 [24 - 156)	33 (9 – 85)

* Derivation set: median [range]; validation set: median [IQR].
Missing data: Obesity n= 71 (9.5); Steroid therapy n = 27 (3.6); Anastomosis n = 34 (4.6)

Table 2. Causes of pouch failure (n=45)

Cause of pouch failure	
Active Crohn's disease in pouch	14
Chronic pouchitis	9
Pouch dysfunction (high defecation frequency, pain, patients' preference)	9
Fistulas*	5
Cancer or dysplasia in the pouch	4
Fecal incontinence	2
Pouch dysfunction due to vasculitis	2

*Related to the pouch, excluding fistulas due to Crohn's

Model performance

The Cleveland Clinic prognostic model includes 4 independent variables: completion proctectomy, handsewn anastomosis, diagnosis of diabetes, and diagnosis of Crohn's disease. For all these variables, Kaplan-Meier curves of our validation cohort are displayed in **Figure 1**. In the present cohort, a trend was shown for better pouch survival in patients who had completion proctectomy compared to patients who had a total proctocolectomy (P=0.053). Patients with a handsewn anastomosis had a significantly worse pouch survival compared to patients with a stapled anastomosis (P=0.022). There was no difference in pouch survival in the patients who had diabetes at the time of surgery or Crohn's disease. The Cleveland Clinics published model including 4 independent variables was externally validated on our validation cohort; results are displayed in **table 3**. Hand sewn anastomosis was the only significant independent predictor. Although not significant, Crohn's disease showed a similarly increased hazard ratio as the Cleveland Clinic study.(1) The Harrell's concordance error rate was 0.42, indicating poor performance of our validation model.

Table 3. Multivariable Cox regression analysis for pouch survival (Cleveland Clinic *vs.* Multicenter cohort)

	Cleveland Clinic		Multicenter cohort	
	Hazard Ratio (95% CI)	P value	Hazard Ratio (95% CI)	P value
Completion proctectomy	1.44 (1.08 - 1.93)	0.018	0.52 (0.26 – 1.02)	0.058
Handsewn anastomosis	1.72 (1.23 - 2.42)	0.003	3.01 (1.04 – 8.64)	0.041
Diabetes	2.67 (1.46 - 4.89)	0.002	0.46 (0.06 – 3.41)	0.453
Crohn's disease	2.37 (1.48 - 3.79)	<0.001	2.03 (0.70 – 5.90)	0.190
The Harrell concordance error rate	**0.32**		**0.42**	

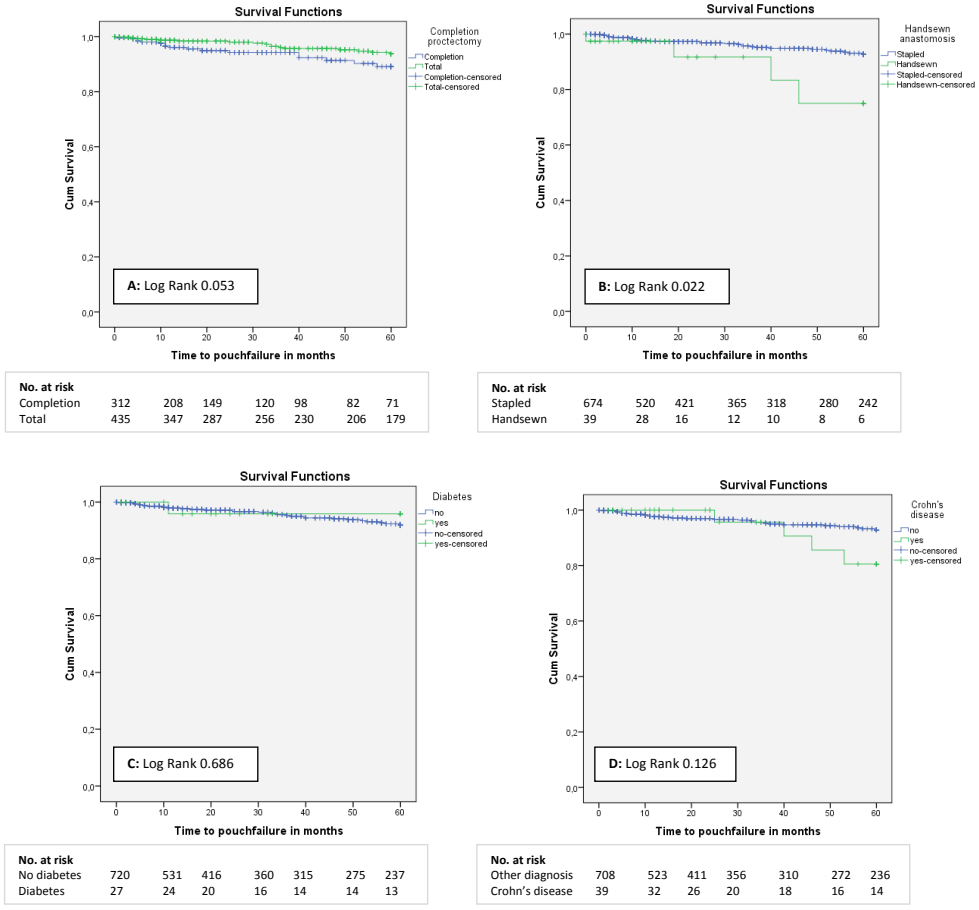

Figure 1. Kaplan Meier analysis for pouch survival comparing completion proctectomy to total proctocolectomy (**A**), handsewn to stapled anastomosis (**B**), patients with diabetes mellitus to all other patients (**C**) and Crohn's disease to other indications for pouch surgery (**D**).

Exploratory analysis

Anastomotic leakage and Crohn's disease of the pouch were strong postoperative predictors for pouch failure and showed a significant difference in pouch survival after 10 years ($p<0.001$), as displayed in a Kaplan-Meier analysis (**Figure 2**).

Univariable cox regression identified 4 variables for inclusion in multivariable analysis; sex, Crohn's disease, handsewn anastomosis and completion proctectomy. Multivariable analysis identified handsewn anastomosis and one stage proctocolectomy as independent risk factors for pouch failure (**table 4**).

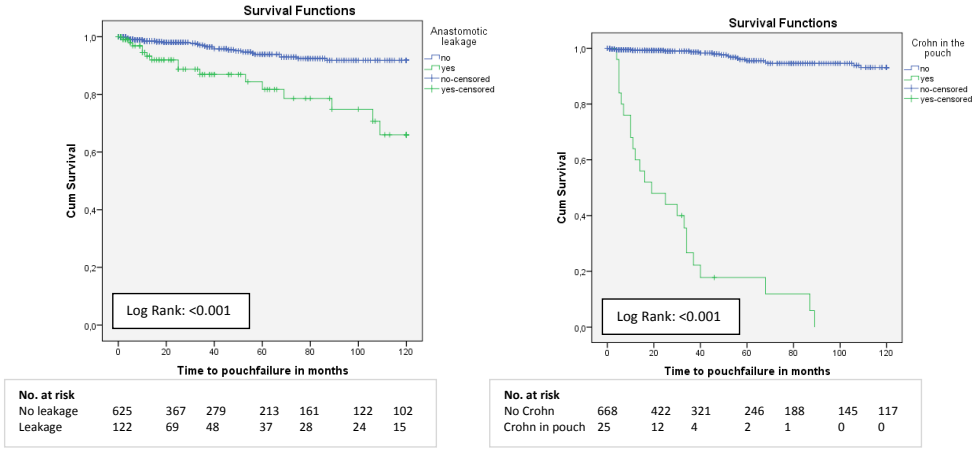

Figure 2. Kaplan Meier analysis for pouch survival after 10 years of follow up, comparing patients with and without anastomotic leakage (A) or Crohn's in the pouch (B).

DISCUSSION

An accurate prediction model is a valuable tool in the everyday clinic for shared decision-making. Internal validation of the Cleveland Clinic model showed a Harrell's concordance error rate of 0.36, indicating moderate performance. Unfortunately, the derivation stage model-building always has the difficulty to overcome over-optimism and bias.(6) In general, external validation of a model will lead to a poorer performance, as was the case in the present study. Application of the proposed Cleveland Clinic model on our multicenter validation cohort yielded a Harrell's concordance error rate of 0.42, indicating poor performance. The only variable that showed a similar hazard ratio as to the proposed Cleveland Clinic model and was significantly associated to pouch failure was handsewn anastomosis. Therefore we feel that this model should not be used to withhold pouch surgery for these patients.

Developing an accurate prediction model remains a difficult task despite a large sample size. We believe that the poor performance of the model in our validation cohort is most likely due to the fact that certain variables were not taken into account. Previous studies have shown that the risk of pouch failure increases significantly in patients with pelvic sepsis.(3,8,9) As shown in our study, we found a strong correlation between anastomotic leakage and pouch failure and Crohn's disease of the pouch and pouch failure. These variables determine the outcome significantly and therefore unable model development for the prediction of pouch failure.

We found that handsewn anastomosis was the only independent risk factor that remained consistently significant between both models. This may be explained by the

fact that in most cases, a handsewn anastomosis is indicated for technically challenging anastomoses with an enhanced risk for pelvic sepsis. In line with this, a previous study by the Cleveland Clinic group showed a significantly higher septic complication rate in patients with handsewn anastomosis compared with stapled anastomosis.(10) Interestingly, by excluding the variables that may have modulated the hazard rates (anastomotic leakage and Crohn's of the pouch cases), we still found that handsewn anastomosis increases the risk for pouch failure. Increased vigilance for complications is needed and should be discussed with the patient in case a handsewn anastomosis is anticipated.

In the current multicenter cohort, patients with a completion proctectomy had a lower risk of pouch failure compared to patients undergoing a total proctocolectomy with IPAA in one stage. These results were within our expectation but in contrast to that of the Cleveland Clinics model.(1) In their study, they actually found a higher risk for pouch failure after completion proctectomy (multistage procedure). The authors argue that their surprise finding may have been due to the fact that patients were in a worse disease state with more florid disease which may have caused technical difficulties during completion proctectomy. We believe that directly creating an IPAA in a severely ill patient leads to a higher major morbidity rate on the short term and subsequently increases the risk for pouch failure.

In the Cleveland Clinic study(1) the postoperative diagnosis of Crohn's disease was used to determine predictive factors for pouch failure. The authors argue that the majority of patients had an initial colectomy followed by IPAA in which the pathology was unknown and that it may have affected their result negatively. Although we do believe that Crohn's disease affects pouch survival adversely, the small proportion of undiagnosed cases in our study did not affect our outcomes. Our experience with patient that had been diagnosed with Crohn's disease before surgery and indicated for a pouch due to quiescent disease not affecting the rectum, have a better outcome than patients diagnosed postoperatively.

In contrast to our study, the Cleveland Clinic study(1) found a worse pouch survival in diabetic patients. It is unknown why these patients have a worse pouch survival compared to the diabetic patients in the multicenter validation cohort since the incidence is approximately the same (3.9% versus 3.6%). We can hypothesize that the small proportion of diabetics in our cohort were adequately treated without end organ failure. Whether long-term effects of diabetes may have the potential to compromise the microvasculature supplying the ileal anal anastomosis or pouch is an interesting question. A large statewide collaborative study of 5123 patients evaluated the anastomotic leak rate and mortality after colectomy in diabetic patients. Although diabetes did not appear to be a risk factor for anastomotic leakage; diabetic patients who had a leak had a 4-fold higher mortality compared with non-diabetic patients.(11) Even though in our study diabetes was not associated with pouch failure, pouch survival rates were considerably decreased in patients that had anastomotic leakage.

Strength and limitations of the study

The main limitation of this validation study is the sample size of the cohort. Although it is a large series of IPAAs, the number of patients with pouch failure within 120 months of follow up was only 45. The power to detect independent predictors in a multivariable analysis is therefore low. However, the 747 patients represent a large IPAA cohort, with over 13 years of clinical practice in dedicated IBD centers, therefore our cohort is a good reflection of daily practice.

The Cleveland IPAA population does not seem to reflect the multicenter cohort when comparing baseline characteristics. In the Cleveland Clinics study, a defunctioning ileostomy is performed routinely, only omitting a stoma in low risk patients. The majority of patients (93.6%) underwent an open procedure and there were less familial adenomatous polyposis patient (6.1%), (table 1). However, we believe that these differences did not influence the performance of the model significantly. Another limitation is that part of the data was collected retrospectively which could possibly lead to a reporting bias. Nevertheless, missing data rates were acceptable.

The poor performance of the Cleveland Clinic prognostic model does not make it suitable for application in daily clinical practice. Based on presently available evidence it is not possible to make clear preoperative recommendations on whether to construct an IPAA or not. However, handsewn anastomosis remained consistently associated with pouch failure in our cohort and should therefore be discussed with the patient. A prediction model for anastomotic leakage or Crohn's of the pouch may be a better solution since these variables are strongly associated to pouch failure.

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CHAPTER 10

Does outcome after restorative proctocolectomy and ileal pouch-anal anastomosis differ between children and adults?

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ABSTRACT

Background: Studies comparing the outcome of ileal pouch-anal anastomosis (IPAA) in children and adults are scarce. This complicates decision making in these young patients. Therefore, we aimed to compare adverse events and pouch function between pediatric and adult patients who underwent IPAA.

Methods: In this cross-sectional cohort study, all consecutive children (<18 years) and adults with a diagnosis of inflammatory bowel disease or familial adenomatous polyposis that underwent IPAA in a tertiary referral center were included (2000–2015). Adverse events were assessed by chart review and pouch function by interview using the Pouch Function Score (PFS).

Results: In total, 445 patients underwent IPAA: 41 pediatric (median age 15 years) and 404 adult patients (median age 39 years), with a median follow of 22 months (IQR 8–68). In pediatric patients, overweight, previous abdominal surgeries, open procedures and defunctioning ileostomy were less prevalent compared to adult patients ($p<0.05$).
The occurrence of anastomotic leakage, surgical fistulas, chronic pouchitis and Crohn's of the pouch was not associated with pediatric age, neither was pouch failure. Pediatric age at time of IPAA was an independent risk factor for developing anastomotic strictures (OR: 4.2 [95%CI: 1.1– 15.8]; $p=0.032$). Current pouch function at last follow-up was similar between pediatric and adult patients (median PFS 5.0 vs. 6.0, $p=0.194$).

Conclusion: Long-term pouch failure rates and pouch function were similar between pediatric and adult patients. There is no need for a more cautious attitude in the application of IPAA in pediatric patients based on concerns of poor outcome.

INTRODUCTION

Restorative proctocolectomy (RPC) with ileal pouch-anal anastomosis (IPAA) is the surgical treatment of choice for therapy refractory ulcerative colitis, and colonic Crohn's disease or inflammatory bowel disease (IBD)-unclassified without evidence of anorectal or ileal disease[1,2]. In addition, RPC with IPAA is the prophylactic treatment for patients diagnosed with familial adenomatous polyposis (FAP)[13]. This procedure has the potential to restore functionality of the resected rectum.

In IBD patients, pediatric-onset disease is described to be more severe with more extensive inflammation, aggressive disease behavior and eventually a higher risk of colectomy compared to adult-onset IBD[4,5]. The 5 year surgery rate in pediatric-onset UC can be as high as 26% compared to adult-onset UC which is approximately 16%[6]. Although colectomy is inevitable and potentially lifesaving in IBD patients with refractory acute severe colitis, the timing of colectomy in patient with chronic ongoing disease is still a topic of discussion[7]. In FAP patients, the onset of polyps has been noted within a broad age range (4 – 35 years)[8] and timing of IPAA surgery has not yet been standardized[9,10]. The decision to operate in pediatric patients with IBD and FAP balances the risk of complications of ongoing disease versus surgical risk and functional implications associated with creating an IPAA as well as the risk of getting a stoma. Families and physicians may therefore be hesitant to decide for IPAA creation during childhood. The main controversy in pediatric patients with an indication for colectomy is timing of RPC with IPAA, either during childhood or delaying the procedure into adulthood.

Recent studies have shown high rates of adverse events[11,12], but satisfactory functional outcomes and quality of life in pediatric patients[13–15], comparable to studies in adults[16,17]. However, there is a paucity of studies directly comparing outcomes of patients which had RPC with IPAA during childhood or adulthood[14,18,19]. Moreover, these studies investigated only patients with IBD[18], reported only short-term outcomes[14] or suffered from small sample sizes[14,19]. This complicates decision making in young patients. Therefore, the aim of this study was to compare adverse events and pouch function in pediatric and adult patients who underwent IPAA surgery.

METHODS

Patients

In this retrospective cohort study, we included all eligible pediatric (< 18 years at pouch surgery) and adult (≥ 18 years at pouch surgery) patients with a diagnosis of IBD (ulcerative colitis, Crohn's disease or IBD-unclassified) or FAP who underwent IPAA surgery in the

Emma Children's Hospital, Academic Medical Center, Amsterdam, the Netherlands between January 2000 and January 2015. Diagnosis of IBD was confirmed by ileocolonoscopy with histologic confirmation. Diagnosis of FAP was established by genetic testing and confirmed by a positive genetic test in more recent cases. Approval from the local Medical Ethics Review Committee was obtained.

IPAA procedure

The AMC is a tertiary referral center for pouch surgery. Ileal pouch-anal anastomosis was performed by the same highly experienced team of colorectal surgeons in all subjects, consisting of pediatric and colorectal surgeons, with at least one of three senior supervising surgeons present during the surgery. Patients were treated by a 1-, (modified) 2-, or 3-stage procedures depending on the diagnosis and the time period[20]. Modified 2-stage procedures were mainly applied in IBD patients based on high risk of anastomotic leak in patients treated with biologicals[20]. The diameter of the stapling gun (29 mm) was identical in pediatric and adult patients. Some of the FAP patients had a mucosectomy and a hand sewn anastomosis, based on the presence of dysplasia in the anal transition zone[21].

Data Collection

Patients who underwent IPAA were identified from a prospectively maintained surgical database. Additional data on patient characteristics and surgical variables were identified by retrospective chart review. Preoperative variables that were extracted were age, gender, preoperative diagnosis, duration of disease course, body mass index (BMI), and the American Society of Anesthesiologists classification (ASA) score. Disease duration was defined as the time between diagnosis and pouch surgery. Overweight was defined as a BMI was > 25 kg/m. The ASA score was dichotomized with a cut-off point of 3, based on previous literature and clinical relevance[22]. Preoperative use of immunomodulators, steroids and anti-tumor necrosis factor alpha (anti-TNFα) were documented. Preoperative steroid use was defined as any use of steroids within 12 weeks prior to surgery. Preoperative anti-TNFα use was defined as infusion within 12 weeks prior to surgery, based on anti-TNFα half-life[23].

Surgical variables included primary proctocolectomy or completion proctectomy, laparoscopic or open approach, type of pouch created, hand-sewn or stapled pouch anal anastomosis, creation of a defunctioning ileostomy, perioperative blood transfusion, duration of surgery and postoperative length of stay. The moment of enrolment during the study period was calculated from January 2000. This was considered relevant since change in management over the years may influence the outcome. Laparoscopic approach was defined as a total laparoscopic IPAA or laparoscopic RPC with hand-assisted laparoscopic IPAA (via Pfannenstiel incision).

Adverse events

Adverse events were anastomotic leakage, fistula related to the surgery, anastomotic stricture, (chronic) pouchitis, Crohn's of the pouch and pouch failure. Anastomotic leakage was defined as any defect at the anastomotic site confirmed on imaging procedures, examination under anesthesia or by surgical re-intervention (requiring radiologic placement of a pelvic drain, trans-anal lavage, endosponge placement or ileostomy creation). A symptomatic stricture at the pouch-anal anastomosis that required dilatation was scored as anastomotic stricture. Crohn's of the pouch was diagnosed if there were non-surgery-related perianal fistulae, granulomas on histology, or inflammation and ulcerations in the afferent limb or in the small bowel on endoscopy in the absence of non-steroidal anti-inflammatory drug use[24]. Pouchitis was diagnosed when the modified Pouchitis Disease Activity Index score was ≥ 5[25]. Pouchitis was sub-classified as chronic pouchitis when patients failed to respond to a 4-week course of a single antibiotic (metronidazole or ciprofloxacin), requiring prolonged therapy of ≥ 4weeks consisting of ≥ 2 antibiotics, oral or topical 5-aminosalicylate, corticosteroid therapy, or oral immunomodulator therapy[26,27]. Pouch failure was defined as formation of a permanent ileostomy, excision of the ileoanal pouch, or pouch-related mortality during the follow-up period.

Functional outcome

All eligible patients were contacted by telephone, up to a maximum of three attempts to answer questions on their pouch function of the last month using the pouch function score (PFS)[28]. The PFS is a seven item scoring system (e.i. 24-hour and nocturnal stool frequency, urgency, major and minor incontinence, antidiarrheal and antibiotic therapy), ranging from 0 – 30 (higher scores indicates worse pouch function), using symptoms that have influence on the quality of live[28].

Statistical Analysis

Primary analysis was the difference in adverse outcomes between patients who had IPAA surgery during child- or adulthood. Secondary analysis was the difference in total PFS and individual PFS components between patients who had IPAA surgery during child- or adulthood. Continuous data with a normal distribution were presented as mean and standard deviation (SD), and T-tests were used. Continuous data with a non-normal distribution were presented as median and interquartile range (IQR), and Mann–Whitney U tests were used. Categorical data were presented as percentages. Fisher's exact tests were used for binary and nominal data and exact linear-by-linear test for ordinal data. Missing data were assumed to be missing at random. Multiple imputation, using a multivariable model with 5 imputations, was performed to adjust for missing values[29]. Univariable and multivariable logistic regression analysis were performed to identify if pediatric age was associated with

adverse outcomes. Variables with a two-sided $p < 0.10$ in univariable regression, were considered for inclusion in multivariable analysis, additionally corrected for the moment of enrolment during the study period. Significance was set at $p < 0.05$. Statistical analysis was performed using IBM SPSS Statistics 22 for Windows.

Table 1. Demographic and surgical characteristics of pediatric and adult patients that underwent IPAA surgery

	Pediatric (n = 41)	Adult (n = 404)	P value
Male (n, %)	23 (56)	219 (54)	0.870
Age at surgery (median, IQR)	15 (13 – 17)	39 (30 - 47)	<0.001
Smoking (n, %)	3 (7)	55 (14)	0.331
Overweight (BMI >25) (n, %)	3 (7)	117 (33)	0.001
Previous abdominal surgery (n, %)	2 (5)	77 (19)	0.019
Diagnosis (n, %)[a]			0.231
Ulcerative colitis	22 (54)	259 (64)	
Indeterminate colitis	3 (7)	44 (11)	
Crohn's disease	2 (5)	13 (3)	
Familial adenomatous polyposis	14 (34)	88 (22)	
ASA score 3 (n, %)	0 (0)	13 (3)	0.620
Immunomodulators ever (n, %)	15 (39)	128 (40)	0.864
Preoperative anti-TNF[a] (n, %)	0 (0)	14 (4)	0.629
Preoperative steroids[a] (n, %)	1 (3)	34 (9)	0.345
Emergency surgery (n, %)	2 (5)	27 (7)	1.000
Completion proctectomy (n, %)	25 (61)	201 (50)	0.192
Primary defunctioning ileostoma (n, %)	4 (10)	109 (27)	0.014
Type pouch (n, %)			0.144
J-Pouch	39 (95)	342 (86)	
Other[b]	2 (5)	66 (16)	
Laparoscopic colectomy (n, %)	38 (93)	223 (55)	<0.001
Hand sewn anastomosis (n, %)	4 (10)	18 (5)	0.142
Length of stay after IPAA (days, median, IQR)	10.0 (8.0 – 14.0)	10.0 (8.0 – 13.0)	0.828
Follow up (months, median, IQR)	21 (6.0 – 50.0)	22.0 (8.0 – 63.5)	0.328
Moment of enrolment during study period (years, median, IQR)[c]	10.0 (5.0 – 13.0)	8.0 (4.0 – 12.0)	0.077

Fisher's exact test: binary and categorical variables; Mann-Whitney U test: continuous variables

[a] < 3 months before surgery

[b] W/B/S pouch

[c] calculated from January 2000

ASA: American Society of Anesthesiologists class, a-TNFα: anti-Tumor Necrosis Factor alpha, BMI: body mass index, CCI: Charlson's Comorbidity Index, IPAA: Ileo Pouch Anal Anastomosis

Variables containing missing data: smoking n = 44 (9.8%), length n = 39 (8.1%), BMI n = 56 (12.6%), steroid therapy n = 28 (5.9%), immunomodulators n = 89 (20.0%), preoperative anti-TNF n = 23 (5.2%), preoperative steroids n = 24 (5.4%), emergency surgery n = 10 (2.2%), type pouch n = 5 (1.1%), handsewn anastomosis n = 20 (4.5%), length of stay after IPAA n = 4 (0.9%).

RESULTS

A total of 445 consecutive patients underwent primary IPAA surgery between January 2000 – 2015, including 41 pediatric (56% male, median age 15 years [IQR 13 – 17]) and 404 adult patients (54% male, median age 39 years [IQR 30 – 47]). The primary diagnosis was similarly distributed between pediatric and adult patients: 281 patients with ulcerative colitis (total 63%; pediatric 54%; adult 64%), 47 patients with IBD-unclassified (total 11%; pediatric 7%; adult 11%), 15 patients with Crohn's disease (total 3%; pediatric 5%; adult 3%) and 102 patients with FAP (total 23%; pediatric 34%; adult 22%). Pediatric patients less frequently had overweight (7% vs. 33%, p = 0.001) or a history of abdominal surgery before restorative proctocolectomy (5% vs. 19%, p = 0.019) compared to adult patients. The procedure was more often performed laparoscopically (93% vs 55%, p < 0.001) and fewer defunctioning ileostoma's were constructed (10% vs. 27%, p = 0.014) in pediatric patients. Time of follow-up and the moment of inclusion during the study period did not differ between children and adults. No pouch related mortality was observed during follow-up. Patient characteristics and surgical details are depicted in **table 1**.

Adverse events

Anastomotic leakage, fistulas, (chronic) pouchitis and Crohn's of the pouch occurred in similar proportions between children and adults. The only significant difference between pediatric and adult patients was found in development of anastomotic strictures, which were more prevalent in pediatric compared to adult patients (10% vs. 3%; p = 0.040). This difference was mainly present in FAP patients (pediatric 21% vs. adult 3%; p = 0.035), and not in IBD patients (pediatric 4% vs. adult 3%; p = 0.526). Pouch failure occurred in 27 patients (6.1%), with no significant difference between pediatric and adult patients, and in FAP or IBD separately. Adverse events rates are shown in **table 2**.

Multivariable analysis demonstrated that IPAA surgery during childhood was not associated with anastomotic leakage, fistulas, chronic pouchitis, Crohn's of the pouch (in IBD patients), neither with pouch failure (**table 3**). Pediatric age at surgery was an independent risk factor for developing anastomotic strictures (OR 4.2 [95% CI 1.1 – 15.8]; p = 0.032), after adjustment for potential confounding variables i.e. type of diagnosis, defunctioning ileostomy, completion protectomy, hand sewn anastomosis, and moment of enrolment during the study period. Anastomotic strictures were successfully treated through a single dilatation (endoscopic or manual) in all pediatric (n = 4) and 73% of adult patients (n = 8). Details on patients with anastomotic strictures are depicted in **supplementary table 1**.

Table 2. Differences in adverse outcomes between pediatric and adult patient

	All (n = 445)		P value	FAP (n = 102)		P value	IBD (n = 339)		P value
	Pediatric (n = 41)	Adult (n = 404)		Pediatric (n = 14)	Adult (n = 88)		Pediatric (n = 27)	Adult (n = 316)	
Anastomotic leakage (n, %)	6 (14)	65 (16)	1.000	2 (14)	16 (18)	1.000	4 (15)	49 (16)	1.000
Pouch stricture (n, %)	4 (10)	11 (3)	0.040	3 (21)	3 (3)	0.033	1 (4)	8 (3)	0.526
Fistulas related to pouch(n,%)	1 (2)	26 (6)	0.496	1 (7)	9 (10)	1.000	0 (0)	21 (7)	0.380
Pouchitis (n, %)	9 (22)	78 (19)	0.681	1 (7)	1 (1)	0.257	8 (30)	77 (24)	0.642
Chronic pouchitis (n, %)	2 (5)	32 (8)	0.757	0 (0)	0 (0)	-	2 (7)	32 (10)	1.000
Crohn's of the pouch (n, %)	-	-	-	-	-	-	4 (15)	19 (6)	0.095
Pouch failure (n, %)	3 (7)	25 (6)	0.735	1 (7)	4 (5)	0.530	2 (7)	22 (7)	0.700

Fisher's exact test was used to calculate differences in adverse events rates between pediatric and adult patients. FAP: Familial Adenomatous Polyposis, IBD: inflammatory bowel disease

Table 3. Multivariable analysis: association between IPAA during childhood and adverse events

	Anastomotic leakage OR (95% CI)	**Pouch stricture** OR (95% CI)	**Fistulas** OR (95% CI)	**Chronic pouchitis** OR (95% CI)	**Crohn's of the pouch**[a] OR (95% CI)	**Pouch failure** OR (95% CI)
Childhood	**0.88 (0.35 – 2.22)**	**4.22 (1.13 – 15.77)**[b]*	**0.63 (0.08 – 5.21)**	**0.58 (0.13 – 2.56)**	**3.07 (0.87 – 10.82)**	**2.24 (0.59 – 8.59)**
IBD-U or CD	-	-	-	-	9.08 (3.68 – 22.41)*	-
IBD	-	-	0.33 (0.13 – 0.83)*	NA	NA	-
ASA score 3	-	-	5.83 (1.45 – 23.52)*	-	-	6.25 (1.59 – 24.61)*
Preoperative steroids	-	-	-	2.52 (0.96 – 6.63)	-	2.05 (0.67 – 6.30)
Preoperative a-TNFα	-	-	-	-	-	-
Completion proctocolectomy	-	3.44 (0.95 – 12.41)*	-	-	-	-
Primary defunctioning ileostomy	-	2.91 (0.91 – 9.32)	2.29 (0.94 – 5.60)	-	-	-
Hand sew anastomosis	-	4.27 (1.08 – 16.93)*	2.32 (0.55 – 9.74)	-	-	-
Laparoscopic procedure	-	-	0.35 (0.14 – 0.87)*	-	-	0.50 (0.21 – 1.20)
J pouch *vs*. other[c]	3.19 (1.12 – 9.06)*	-	-	-	-	-
Time diagnosis - IPAA (months)	1.00 (1.00 – 1.01)*	-	-	-	-	-

* Significantly associated with outcome in multivariable regression analysis, corrected for the moment of enrolment during the study period (calculated from January 1991). Variables with a p value of ≥ 0.1 in univariable analysis are indicated by (-) and excluded from multivariable analysis. The factor childhood was adjusted for all variables with a p value of < 0.1.

[a] in IBD patients only (n = 339)

[b] additional correction for type of diagnosis: IBD vs. FAP

[c] W/B/S pouch

ASA: American Society of Anesthesiologists class, a-TNFα: anti-Tumor Necrosis Factor alpha, BMI: body mass index, CCI: Charlson's Comorbidity Index, CD: Crohn's disease, IBD: inflammatory bowel disease, IBD-U: inflammatory bowel disease unclassified, IPAA: Ileo Pouch Anal Anastomosis

Pouch function score

In total, 29 pediatric (78% of 38 eligible patients) and 253 adult patients (70% of 363 eligible patients) completed the pouch function score. In pediatric patients that completed the PFS, overweight, laparoscopic colectomy and completion proctectomy were more prevalent, and the moment of enrolment was later during the study period compared to adult patients (**supplementary table 1**).

Patients that underwent IPAA surgery during childhood had a lower 24-hour stool frequency compared to patient that had surgery during adulthood (p = 0.008). There was no difference in nocturnal stool frequencies, urgency for defecation and incontinence between patients who had surgery during child- or adulthood (**table 4**). Antidiarrheal medication use was less frequent in children compared to adults (31% vs 55%; p = 0.018). Antibiotic treatment for pouchitis was more frequently used in children compared to adults (24% vs 7%; p = 0.006). There was no difference in total pouch function score between children compared to adults (**table 4**).

DISCUSSION

The objective of the current study was to identify whether the outcome of ileo-anal pouch surgery is affected by the age of IPAA construction. Pediatric age was not associated with most well-known adverse events after IPAA creation, such as anastomotic leakage, surgical fistulas, pouchitis and Crohn's of the pouch. Nonetheless, we identified pediatric age at surgery as an independent risk factor for anastomotic strictures of the pouch. Long-term pouch failure rates and pouch function were similar between patients who underwent surgery during child- and adulthood.

Parks et al. first described the IPAA in 1978[30], where after first pediatric series emerged in the early nineties[31]. Since then, only three studies have been published assessing postoperative outcomes and debating whether surgery should be postponed until adulthood[14,18,19]. An important factor that may influence the pouch functionality is the (changing) anatomy during the adolescent growth period. In both male and female adolescents, the pelvis increases in width while longitudinal growth ceases[32–34]. In addition, more severe disease characteristics in young patients with IBD[4,5], may influence the outcome of IPAA surgery. In line with previous studies, anastomotic leakage[14,18], fistula related to surgery[18] and chronic pouchitis[14,18] were not associated with pediatric age. Furthermore, pouch failure rate was similar between children and adults (7% vs. 6%) and corresponded to a recent meta-analysis of adult patients with established ulcerative colitis or FAP (4.3% [95% CI, 3.5–6.3])[16].

Strictures of the pouch, which all occurred at the level of the pouch-anal anastomosis, were associated with a pediatric age at IPAA surgery, merely due to the higher anastomotic

stricture rate in pediatric FAP patients. In a previous cohort of patients with IBD, however, pouch stricture also occurred more frequently in pediatric (12.5%) compared to adult patients (5.8%, p = 0.008).[18] Various risk factors have been associated with the occurrence of pouch strictures such as a hand sewn technique of the IPAA[35], small diameter of the stapling gun, a defunctioning ileostomy, anastomotic dehiscence and pelvic sepsis[36]. As expected, this study showed a significant association between pouch strictures and defunctioning ileostomy, completion proctocolectomy and hand-sewn anastomosis and these factors were therefore adjusted for in multivariable analysis. Furthermore, the rate of anastomotic leakage and the diameter of the staple gun were equal, hence, could not affect the association between surgery during childhood and anastomotic stricture. It remains speculative why younger age is associated with more strictures. One of the hypotheses may be a greater risk of mucosal tears when inserting the circular stapler in children, resulting in more anastomotic strictures.

Fortunately, treatment of anastomotic strictures in clinical practice is merely successful. In this cohort, all anastomotic strictures in pediatric patients were treated successfully with a single dilatation. In a large cohort (N = 213) of patients with IBD and FAP that developed anastomotic strictures, the majority of strictures required only a single dilatation as well(88%)[35]. Recurring strictures may necessitate excision of the fibrotic ring, and corresponding advancement of the ileal mucosa to bridge the anastomotic mucosal gap[35]. However, routine digital and, if necessary, proctoscopic assessment of the pouch at the initial postoperative visit or at the time of the ileostomy closure could identify early non-symptomatic stricture of the anastomosis and may potentially avert more invasive treatment.

A good long-term pouch function is an important argument to comfort young patients who are facing pouch surgery and is strongly associated with health-related quality of life.[28,37]. In the current cohort, the overall pouch function was comparable between pediatric and adult patients and equivalent to a cohort of 196 adults with IBD (median PFS, 6/30 [IQR 3–11])[37]. Interestingly, pediatric patients in this cohort exhibited a lower 24-hour stool frequency compared to adults. The lower stool frequency, together with less frequent use of antidiarrheal medication in pediatric patients, may indicate a higher adaptive ability of the pouch during childhood. Treating physicians should, however, keep in mind that these results apply to patients treated within a specialized center for IPAA surgery. The strong association between reoperation or excision of the pouch and the volume of IPAA surgeries per hospital[38], indicates that both pediatric and adult patients requiring IPAA should be treated in specialized centers.

Legitimate comparability of pediatric and adult patients in this cohort was effectuated by a similar distribution of patient enrollment across the study period and the same team of colorectal surgeons that performed RCP and IPAA. A limitation of this study is the

retrospective nature, which may have led to bias even though only objective and profound adverse events were collected. Furthermore, patients in this study were nationally referred to our specialized pouch center, which may have resulted in patients with a more complex or advanced disease stage, making referral bias inevitable. Most importantly, the low number of adverse events hampers proper correction of the association between pediatric age and anastomotic stricture, found in this cohort, with potential confounders.

In conclusion, long-term pouch failure rates and pouch function were comparable for pediatric and adult patients, despite the association between pediatric age and anastomotic strictures, and provided evidence that pediatric patients with an indication for RPC and subsequently IPAA can expect good pouch outcomes. There is no need for a more cautious attitude in the application of RPC and IPAA in pediatric patients based on concerns of poor outcome on the longer term.

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Supplementary table 1. Characteristics of patients with a pouch stricture

	Pediatric (n = 4)	Adult (n = 11)
Age at surgery (median, range)	14 (12 – 17)	40 (24 – 46)
Familial adenomatous polyposis	3 (75)	3 (27)
Anastomotic leakage	0 (0)	1 (9)
Completion proctectomy	2 (50)	2 (18)
Primary defunctioning ileostomy	0 (0)	7 (64)
Hand sew anastomosis	2 (50)	2 (20)

Supplementary table 2. Differences in demographic and surgical characteristics between pediatric adult patients that completed the pouch function score

	Pediatric (n = 29)	Adult (n = 253)	P value
Male (n, %)[a]	17 (59)	140 (55)	0.844
Age at surgery (median, IQR)	15 (13 – 16)	39 (30 - 48)	<0.001
Smoking (n, %)	2 (7)	34 (14)	0.391
Overweight (BMI >25) (n, %)	3 (11)	81 (36)	0.006
Previous abdominal surgery (n, %)	2 (7)	44 (17)	0.189
Diagnosis (n, %)[a]			0.775
Ulcerative colitis	18 (62)	166 (66)	
Indeterminate colitis	2 (7)	21 (9)	
Crohn's disease	1 (3)	5 (2)	
Familial adenomatous polyposis	8 (28)	60 (24)	
ASA score 3 (n, %)	0 (0)	5 (2)	1.000
Immunomodulators ever (n, %)	11 (41)	84 (41)	1.000
Preoperative anti-TNF (n, %)	0 (0)	9 (4)	0.606
Preoperative steroids (n, %)	0 (0)	18 (7)	0.233
Emergency surgery (n, %)	2 (7)	20 (8)	1.000
Completion proctectomy (n, %)	23 (79)	126 (50)	0.003
Primary defunctioning ileostoma (n, %)	4 (14)	59 (23)	0.347
Type pouch (n, %)			0.549
J-Pouch	27 (83)	219 (88)	
Other[a]	2 (7)	30 (12)	
Laparoscopic colectomy (n, %)	28 (97)	154 (61)	<0.001
Hand sewn anastomosis (n, %)	3 (11)	13 (5)	0.223
Length of stay after IPAA (days, median, IQR)[a]	9.5 (8.0 – 13.0)	10.0 (8.0 – 13.0)	0.803
Follow up (months, median, IQR)	19.0 (4.0 – 45.5)	22.0 (8.0 – 65.0)	0.261
Moment of enrolment during study period (years, median, IQR)[b]	11.0 (8.0 – 13.0)	9.0 (4.0 – 12.0)	0.032

Fisher's exact test: binary and categorical variables; Mann-Whitney U test: continuous variables
[a] W/B/S pouch
[b] calculated from January 1991
ASA: American Society of Anesthesiologists class, a-TNFα: anti-Tumor Necrosis Factor alpha, BMI: body mass index, CCI: Charlson's Comorbidity Index, IPAA: Ileo Pouch Anal Anastomosis

CHAPTER 11

Incidence and Severity of Pre-pouch Ileitis: a Distinct Disease Entity or a Manifestation of Refractory Pouchitis?

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ABSTRACT

Background: Restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) is the operation of choice for patients with treatment-refractory ulcerative colitis (UC). However, following this intervention, up to 50% of patients subsequently develop pouchitis. Moreover, a subgroup will also develop inflammation in the afferent ileum proximal to the pouch, a condition named pre-pouch ileitis (PI).

Methods: Data on 546 patients who underwent IPAA for UC was retrospectively collected from three tertiary inflammatory bowel disease referral centers in the Netherlands, Belgium and England. PI was considered present if there was endoscopic and histological inflammation in the afferent limb proximal to the pouch. Crohn's disease was excluded by assessing the histology of colectomy resection specimens.

Results: PI was present in 33/546 (6%) patients and all of these had concurrent pouchitis. 144 (26%) patients had pouchitis without PI and 369 (68%) patients did not have inflammatory pouch disease. Of the 33 patients with PI 6 (18%) received no specific treatment, 9 (27%) responded to antibiotics and 18 (54%) required escalation in therapy to steroids/ immunomodulators or anti-TNF agents. Potent immunosuppressive treatment was required more frequently in patients with PI than those with pouchitis alone.

Conclusions: Pre-pouch ileitis is a much less common and more treatment refractory condition than pouchitis alone. Once PI is diagnosed, clinicians should be aware that response to antibiotic therapy is less likely than in pouchitis alone. Immunomodulatory therapy and escalation to anti-TNF agents should be considered early in cases of non-response. The suggestion that PI represents misdiagnosed Crohn's disease could not be substantiated in our cohort.

INTRODUCTION

Restorative proctocolectomy (RPC) with ileal pouch-anal anastomosis (IPAA) is the operation of choice for patients with ulcerative colitis (UC) that is refractory to medical treatment and in those who develop dysplasia or cancer. In population based cohort studies this becomes necessary in 10 – 30% of patients after a decade of disease[1-3]. The most common complication following IPAA is pouchitis, with a reported cumulative incidence between 24 – 59%[4, 5]. Moreover, a subgroup will develop inflammation in the afferent limb of the pouch (**figure 1**), which has previously been described as pre-pouch ileitis (PI). This pattern of inflammation can extend for a significant distance into the afferent limb (up to 50 cm)[6] and can include erosions (demonstrated in **figure 2**), ulcerations, erythema and friability. Evidence suggests that this phenomenon is linked to the underlying pathophysiology of inflammatory bowel disease (IBD), as it is virtually non-existent amongst patients undergoing RPC and IPAA for familial adenomatous polyposis (FAP)[6]. The location and appearance of inflammation in PI can closely resemble that of Crohn's disease (CD). Because of this, some clinicians have suggested to reclassify these patients as having CD, rather than UC, once this specific pattern of inflammation is observed[7, 8]. However, this alteration may be errant, as PI has distinct histological changes and pathogenic processes when compared with CD[6]. It has also been demonstrated that PI shares certain morphological changes with those seen in pouchitis[9].

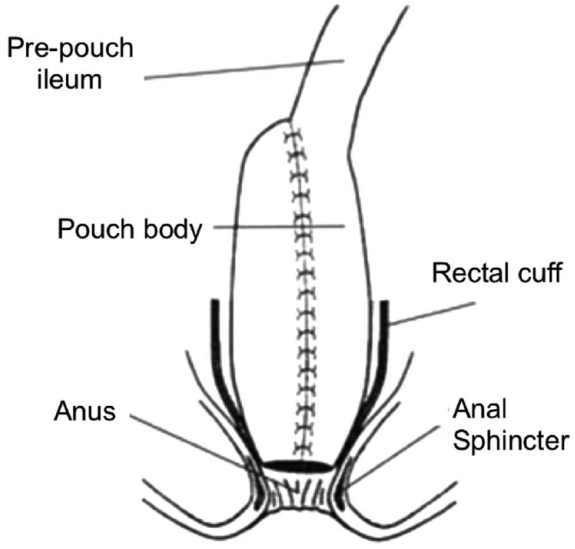

Figure 1. Diagram demonstrating the different anatomic parts of an ileal pouch anal anastomosis

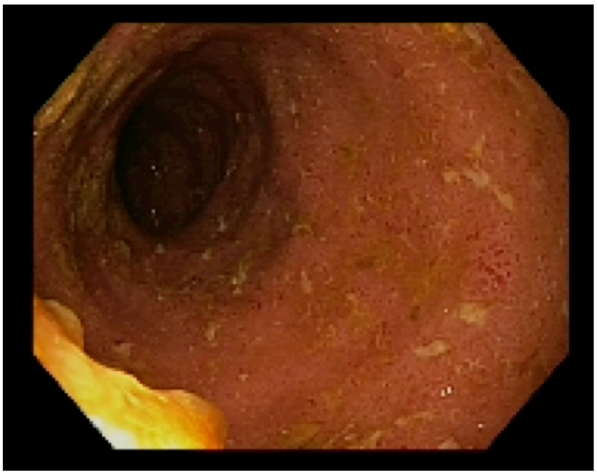

Figure 2. Endoscopic image demonstrating the erythema and erosions that can be seen in pre-pouch ileitis

Primary sclerosing cholangitis (PSC) is the most common hepatobilliary extra intestinal manifestation (EIM) in IBD patients, with a preponderance to UC[10]. It is well recognized that UC-PSC patients exhibit a specific and distinct pattern of the disease characterized by increased incidence of backwash ileitis, rectal sparing, pancolitis, colitis-associated neoplasia, and poorer overall survival[11, 12]. In addition, following IPAA they are known to have an increased risk of developing pouchitis[13] and more recently, an increased risk of PI has also been demonstrated[14]. An underlying autoimmune pathogenesis has been postulated for this association, based on the finding that autoimmune diseases are also more common amongst this group.

The findings discussed above have resulted in the generation of opposing hypotheses regarding PI. Some suggest that it is the same disease process occurring in a different portion of the ileal mucosa (or simply extension of existing pouchitis), whilst others believe it to be a distinct disease entity [6].

There is a relative paucity of knowledge regarding PI when compared to pouchitis as it is less well defined and occurs more rarely. It is also a diagnosis that can easily be missed on account of the fact that it is accepted practice to treat symptoms of pouch dysfunction with empirical courses of antibiotics, without performing endoscopic inspection of the pouch. Even amongst those who do undergo pouchoscopy, the diagnosis could be overlooked if the afferent ileal limb is not intubated and carefully examined with consideration given to taking biopsy samples. We hypothesize that recognition of this disease is relevant as the inflammation involved is more extensive and may be more treatment refractory than pouchitis alone. We therefore sought to characterize this condition by analyzing the ways in which it differs from pouchitis including its incidence, predictive factors and response to treatment.

MATERIALS & METHODS

Identification of patients with an ileal-pouch anal anastomosis (IPAA)

In this retrospective cohort study, data was collected from a prospectively maintained database of 621 consecutive IBD (CD, UC or IBD-unclassified) patients who underwent RPC and IPAA in three tertiary referral centres, the Academic Medical Centre (AMC), Amsterdam, The Netherlands, University Hospital Leuven (UZL), Belgium and University College London Hospital (UCL), London, United Kingdom. The data collection period extended from September 1990 to December 2014. Of the pouch patients records that were screened, 546 were identified who underwent RPC and IPAA for UC, which had been confirmed at the time by clinical, endoscopic and histologic criteria. We reviewed histology reports of biopsy samples and colectomy specimens to reconfirm UC as the underlying diagnosis. Patients with CD or IBDU (inflammatory bowel disease unclassified) were excluded from this study. Endoscopic and histological records were then used to identify individuals affected by PI and pouchitis. Data regarding the surgical construction of the pouch (J, S or W-formations and hand-sewn or stapled) was not collected.

Definition of pre-pouch ileitis (PI)

There is currently no validated definition for PI and due to the retrospective nature of this study, previously suggested definitions for pouchitis[15] could not be adapted for use either. Instead, a similar approach to that used by Shen and colleagues[14, 1614, 16] for defining PI was adopted[14, 16]. This includes the combination of active inflammation at endoscopy with confirmatory histological findings in biopsy samples, but without an objective symptom score. Endoscopy reports were reviewed to identify cases with evidence of macroscopic inflammation in the afferent limb, defined as the presence of any of the following findings: ulcerations, erosions, erythema, friability or haemorrhage. However, due to the fact that this is a retrospective review we could not determine the proximal extent of the inflammation in the afferent limb seen at endoscopy. The presence of active inflammation was confirmed by reviewing histology reports of biopsies taken from the afferent limb during the procedure. If the histology report described acute or chronic active inflammation, PI was considered present. The retrospective design also meant that infective pathogens could not be excluded in all cases by stool sampling but no cases of CMV infection were reported on histology.

After identification of the patients, we collected clinical data through review of the electronic charts. The data was then analysed for differences between the IPAA patients with pouchitis with or without PI. Differences between PI patients and a control group of patients with no pouch inflammation were also investigated. For clarity, the term “pouchitis group” will be used in reference to the group of patients who were diagnosed with pouchitis

in the absence of PI. General demographic data was collected along with data regarding EIM, which were considered present if there was arthritis, uveitis, pyoderma gangrenosum, erythema nodosum, oral ulceration, anal fissures or fistulas. Despite being a hepatobilliary EIM, PSC was considered separately due to its previously demonstrated link with PI[14]. Data on the indication for RPC, the interval between UC diagnosis and IPAA as well as the interval between IPAA and onset of PI were also collected. Backwash ileitis was considered present if there was macroscopic and microscopic evidence of inflammation (including information gained from the subsequent colectomy specimen. This finding is considered by some to be the pre-operative corollary of PI. Finally, the necessity for and response to treatment were investigated by reviewing clinical notes, prescriptions and correspondence on an electronic patient information management system. The use of antibiotic courses (including available information regarding duration and frequency), 5-aminosalicylates, steroids, immunomodulators and anti-TNF treatment was recorded. Data regarding rectally administered medications was not included in our study. Response to treatment was defined in accordance with those previously described for pouchitis[14, 17]. Pouchitis was considered antibiotic responsive when an episode of pouchitis was successfully treated with a two week course of antibiotics. We considered patients antibiotic-dependent if they required multiple (> 4 per year) or continuous courses of antibiotics. Antibiotic refractory pouchitis was considered present when symptoms failed to respond to conventional treatment with antibiotics and required treatment escalation to 5-aminosalicylates, corticosteroids, immunomodulatory therapy, or anti-TNF agents.

Statistical analysis

Groups of patients with, and without PI were compared using Pearson Chi-Square test and the unpaired t-test. Statistical Package for Social Sciences version 22 (SPSS; IBM, Armonk, USA) was used with a pre-defined two-sided significance limit of $P < 0.05$.

RESULTS

Of the 546 UC patients reviewed, 33 were identified with the combination of endoscopic and histological inflammation in the afferent limb, meeting our diagnostic criteria for PI. This represented 6.1% of the total cohort of UC patients, a proportion that is in accordance with previously reported case series[9]. All 33 of these patients also had concurrent pouchitis based on similar endoscopic and histological criteria to those used to define PI. 144 (26.2%) patients had pouchitis in the absence of PI. The remaining 369 (67.7%) UC patients who underwent RPC and IPAA did not develop any subsequent documented episodes of pouch inflammation during the follow-up period of this study and served as our control group (**figure 3**).

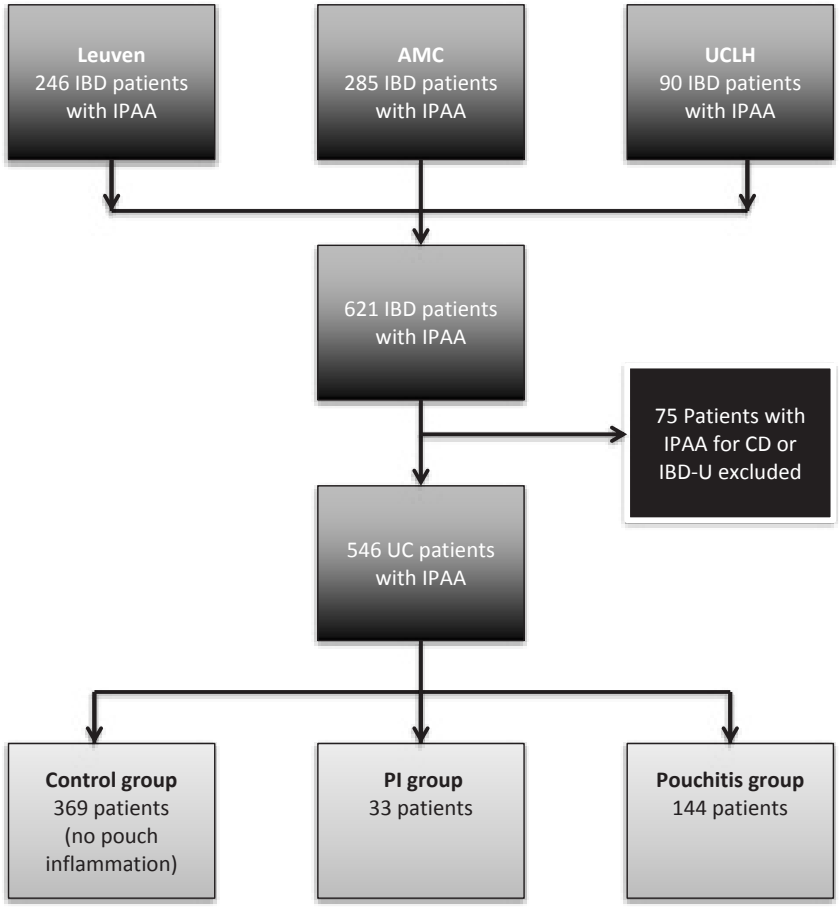

Figure 3. Flow chart demonstrating patient recruitment and allocation

Pre-pouch ileitis compared to control patients

The mean age of the PI group at the time of UC diagnosis was significantly lower than the mean age of the control group (25.4 ± 8.6 versus 32.4 ± 12.5, P = 0.004). Patients who went on to develop PI were also significantly younger when they came to colectomy (33.4 ± 10.5 versus 39.7 ± 13.2, P = 0.009). Though no significant differences were seen based on gender or smoking habits, significantly more patients in the PI group had PSC than in the control group (18% versus 7%, P = 0.025). The finding of backwash ileitis was more frequently seen amongst the PI cohort (5, 19%) compared to the control group (15, 6%, P = 0.020). These results are summarised in **table 1**.

Table 1. Table comparing the demographics and disease characteristics of the control group (patients with no inflammatory pouch disease) with the PI group

	PI	Control Group	*P*
No. patients	33	369	
Gender (female/male)	11/22 (33%/67%)	157/212 (43%/57%)	0.142
Mean age at UC diagnosis, years (±SD)	25.4 (8.6)	32.4 (12.5)	0.004[a]
Mean age at surgery, years (±SD)	33.4 (10.5)	39.7 (13.2)	0.009[a]
Mean interval between diagnosis and surgery, years (±SD)	7.6 (6.4)	7.8 (8.4)	0.903
Smoker	4/27 (15%)	33/308 (11%)	0.515
PSC	6/33 (18%)	26/369 (7%)	0.034[a]
Backwash ileitis	5/26 (19%)	15/234 (6.4%)	0.020[a]
Indication for colectomy			0.239
Therapy refractory	21 (64%)	258 (70%)	
Steroid dependent	4 (12%)	30 (8%)	
Dysplasia/cancer	5 (15%)	35 (10%)	
Other/unknown	3 (9%)	46 (12%)	

[a]Denotes that *P* value reaches statistical significance

Pre-pouch ileitis compared to pouchitis

The mean age at UC diagnosis of the PI group lower than the mean age of the pouchitis group (25.4 ± 8.6 versus 30.1 ± 11.7, P=0.043). 18% of PI patients had concurrent PSC compared with 6% in the pouchitis group (P = 0.027). Backwash ileitis prior to colectomy was found in 19% of the PI patients, compared to only 9% in the pouchitis cohort. However, this finding was not significant (P = 0.138).

No significant differences were found between PI and pouchitis groups with regard to gender, the interval between UC diagnosis and surgery or the interval between pouch construction and the onset of pouchitis.

The indications for surgery were similar in both groups; the majority of patients underwent RPC and IPAA due to therapy refractory UC in the PI and pouchitis group (64% versus 69%). Other indications included steroid dependent UC (12% versus 10%), dysplasia or colorectal cancer (15% versus 11%) and other causes (9% versus 10%) such as side effects of medication (preventing their use to control disease) or toxic megacolon (**table 2**).

Table 2. Table comparing the demographics, disease characteristics and treatment of the pouchitis group with the PI group

	PI	Pouchitis	*P*
No. patients	33	144	
Gender (female/male)	11/22 (33%/66%)	68/74 (48%/51%)	0.121
Mean age at UC diagnosis, years (±SD)	25.4 (8.6)	30.1 (11.7)	0.043[a]
Mean interval between UC diagnosis and surgery, years (SD)	7.6 (6.4)	6.8(6.8)	0.896
Mean interval between surgery and pouchitis, years (± SD)	2.4 (2.9)	2.5 (2.8)	0.653
Backwash ileitis	5/26 (19%)	10/110 (9%)	0.138
PSC	6/33 (18%)	9/143 (6%)	0.027[a]
Indication for colectomy			0.335
Therapy refractory	21 (64%)	94 (65%)	
Steroid dependent	4 (12%)	14 (10%)	
Dysplasia/cancer	5 (15%)	15 (10%)	
Other/unknown	3 (9%)	21 (15%)	
Most potent treatment required			
No treatment	6 (18%)	47 (33%)	0.093
Antibiotics	9 (27%)	79 (55%)	0.004[a]
Steroids or immunomodulators	6 (18%)	9 (6%)	0.029[a]
Anti-TNF	12 (36%)	9 (6%)	<0.001[a]

[a]Denotes that *P* value reaches statistical significance

Treatment

When considering the most potent therapy patients required for their disease, we found that for 78 (55%) patients in the pouchitis group antibiotic treatment was sufficient. 68 of these were antibiotic responsive and 10 were antibiotic dependent (as previously defined[16]). However, in the PI group disease control was attained significantly less frequently with antibiotics alone (9 patients or 27%, P = 0.004) and the remainder required more potent anti-inflammatory treatment. Of those 9 patients 6 (18%) were antibiotic responsive and 3 were antibiotic dependent.

Six (18%) PI patients required steroids or immunomodulatory therapy to control their disease, compared to 9 (6%) pouchitis patients (P=0.029). The remaining 12 (36%) PI patients required escalation to biological treatment, compared to 8 (6%) pouchitis patients in an attempt to achieve a response (P < 0.001) (**Figure 4 and 5**).

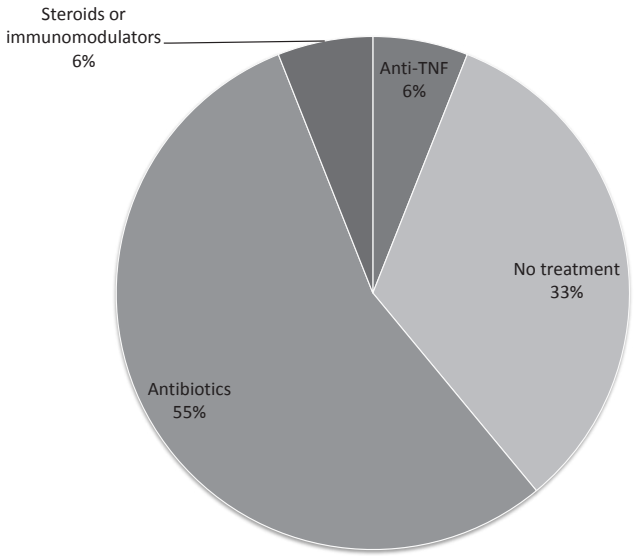

Figure 4. Pie-chart demonstrating the most potent treatment required to treat patients with pouchitis

For 6 (18%) PI patients and 47 (33%) pouchitis patients, with limited symptom burden, a conservative approach was adopted and they did not receive systemic treatment according to their medical records (P=0.093). However, these patients may have been adequately treated with rectally administered topical treatment, as data regarding this route of administration was not collected.

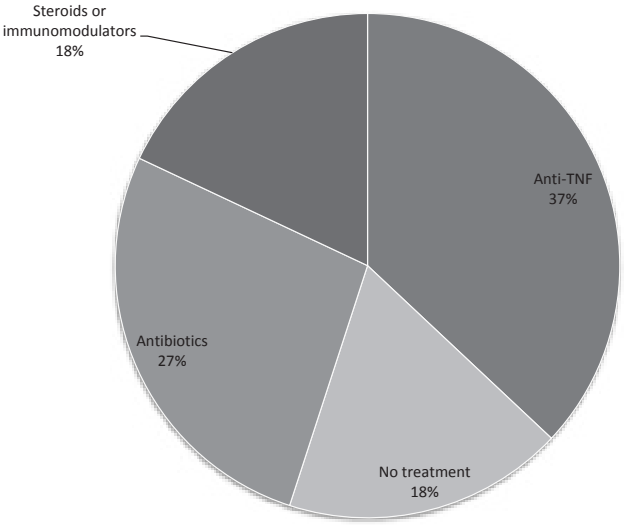

Figure 5. Pie-chart demonstrating the most potent treatment required to treat patients with pre-pouch ileitis

DISCUSSION

Except for the rare finding of backwash ileitis, ileal inflammation is not a recognized feature of UC. However, since the advent of RPC with IPAA, inflammation of the ileal reservoir or 'pouchitis' has been increasingly recognized, characterized and studied. Subsequently, the occurrence of inflammation in the portion of ileum proximal to the pouch has been reported and described as pre-pouch ileitis. It is increasingly being understood that this pattern of disease does not necessarily represent a previous misdiagnosis of Crohn's disease[9]. However, understanding of factors that predict the development of PI is limited and its response to conventional therapy has not yet been well studied. In an attempt to explore these factors, further characterize this relatively novel condition and its response treatment, we compared a group of patients with PI to a control group (no pouch inflammation) and those with pouchitis.

The incidence of PI in our multicenter, retrospective study (6%) is in keeping with that of another single center study, in which an incidence of 5.7% was reported[9]. Pouchitis was also present in similar numbers to previously published cohorts. We found that patients who developed PI had an earlier onset of UC and age at colectomy than those in our control group. These findings may suggest a more aggressive disease pattern. Furthermore, these patients appear to have a more severe inflammatory disease, which often required more potent anti-inflammatory treatments than those with pouchitis alone. Our cohort demonstrated this with significantly higher rates of immunosuppressive and anti-TNF usage amongst patients with PI than pouchitis alone. Although the use of anti-TNF agents in this setting has not been extensively investigated, a small open-label study using infliximab to treat 10 patients with PI and concurrent pouchitis demonstrated good efficacy[18]. After a standard 6 week induction regimen, 8 had complete resolution of endoscopic lesions, 1 demonstrated improvement and 1 showed no change. Moreover, the 8 responders remained in clinical and endoscopic remission at 6 months, without the need for maintenance treatment.

The theory, that PI may in fact be an extension of the inflammatory process seen in pouchitis is also supported by the finding that all patients in our PI cohort had concurrent pouchitis. However, what factors underly this more extensive and/or aggressive disease course remain unclear. Bacterial overgrowth in the afferent limb may be a factor contributing to the development of PI, although evidence of significant obstruction or stricture between the afferent limb and the pouch was not observed in any patients in our PI cohort. Whilst being detrimental in CD, smoking has been shown to be a beneficial in UC and a protective factor against the development of pouchitis[19]. However, we did not find evidence that it significantly alters the development of PI.

We defined PI as the presence of both endoscopic and histological inflammation in the neo-terminal ileum. However, an additional 6 patients were identified in whom there was

histological but not endoscopic inflammation. This may indicate that before macroscopic inflammation is visible at endoscopy, there are already inflammatory processes ongoing at a microscopic level that may lead to PI. Unfortunately, these patients were not routinely followed-up with serial endoscopies as the clinical need did not exist and we are therefore unable to confirm that these findings pre-date macroscopic inflammatory lesions. A limitation of our study is that we did not account for the extent and severity of the inflammation seen at endoscopy. This type of finding would add weight to the concept that PI may be an extensive and more severe form of pouchitis. The afferent limb was not always visualized at endoscopy and even in cases where it was, biopsies were not always taken. In those cases our chosen definition for PI could not be satisfied. This could lead to an underestimation of the actual number of PI patients in our cohort. Due to the retrospective nature of this work, the supervising clinicians impression of therapeutic efficacy was used to judge response and this, as well as being subjective, is open to bias.

The evidence gathered by our own study means that clinicians should be mindful of PI in the context of chronic refractory pouchitis, since this could be a contributory factor to pouch failure[18]. Pouchoscopy with careful examination of the pre-pouch ileum and biopsy samples from this region should be considered in all patients with chronic or refractory symptoms consistent with pouchitis. Once a diagnosis of PI is made, clinicians should commence immunomodulatory therapy earlier in the disease course and consider escalating to an anti-TNF if this proves ineffective.

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Summary and future perspectives

Summary and future perspectives

SUMMARY

Ulcerative colitis is a dreadful inflammatory bowel disease that poses a formidable burden on the lives of young adults. The clinical course is unpredictable, marked by alternating periods of exacerbation and remission. The precise aetiology has not been fully revealed, however, it is widely presumed that mucosal inflammation is caused by a dysregulated immune response to commensal gut flora in a genetically susceptible host. The current treatment guidelines propose a step-up approach starting with the most conservative medication, mainly with 5-aminosalicylic acid (5-ASA) and corticosteroids, to more immunosuppressive drugs and targeted therapies such as anti-TNF and integrin antibodies. Despite significant advances in medical treatment, up to 25% of UC patients eventually require surgery with proctocolectomy and pouch formation. Although the vermiform appendix was generally regarded to be an evolutionary remnant, a substantial body of evidence has accumulated supporting its role in the development and course of UC. This thesis aims to investigate what the role of the appendix is in IBD, evaluate the natural history of UC, and demonstrate risk factors for postoperative complications after pouch surgery.

Part I: The effect of appendectomy in ulcerative colitis patients, experimental and clinical studies

Chapter 1 is a comprehensive overview of the current knowledge about the clinical and immunological aspects of the vermiform appendix in IBD. Clinical studies were systematically reviewed and meta-analysed. A total of thirty-eight case control studies were included evaluating the role of an appendectomy in the prevention of IBD. The majority of these studies showed a significant inverse association between an appendectomy and the development of UC with an overall odds ratio of 0.39 (95% CI 0.29 – 0.52). Four large population based studies evaluated the incidence rate ratios of UC after appendectomy, of which 3 showed a significant lower UC incidence rate after appendectomized patients compared to control patients. Six observational studies were included regarding the therapeutic effect of appendectomy on the course of UC. Although a few studies found no effect, the majority of these studies showed that previous appendectomy patients had a lower relapse rate, less steroid use and a decreased risk of colectomy. Although the molecular mechanism of the link between appendectomy and UC has not been resolved, several theories were proposed that involved both influence on the composition of the microbiota and homing of different immune cell populations. Chapter 2 further describes the current understanding regarding the histology, physiology, and immunological role of the vermiform appendix in health and disease. The importance of the appendix in the development and preservation of Gut Associated Lymphoid Tissue (GALT) and its biofilm is described. Furthermore, evidence

elaborated in this review support the idea that a defective function and interaction with gut flora in the appendix play an essential role in the aetiology and probably also in the onset of UC.

The early results from the PASSION study suggest that UC patients with mucosal appendicitis may benefit from appendectomy (Chapter 3). Patients with therapy refractory UC that were referred for proctocolectomy were invited to undergo laparoscopic appendectomy. Clinical response, remission, failure and pathologic response were determined after 3 and 12 months. In total, 30 patients underwent appendectomy with a mean preoperative total Mayo score of 9 (SD 2). After 3 months, pathological response was seen in 11/23 patients. Active appendiceal inflammation was highly predictive of pathological response when compared to no inflammation or extensive ulcerations. After 12 months, 11 (37%) patients failed, 9 (36%) had lasting clinical response of whom 5 (23%) were in remission. However, a longer follow up is warranted to exclude a possible placebo effect.

If an appendiceal phenotype could be determined predicting clinical response to appendectomy, this could be used to identify patients benefitting from resection. Chapter 4 demonstrated an interesting new concept in which appendiceal lymphocyte infiltration was determined by analysis of appendiceal lavage fluid. The appendix of 41 (15 UC, 9 Crohn's disease, 7 acute appendicitis, 10 non-inflammatory controls) patients was surgically removed during laparoscopy and flushed with 2cc of phosphate buffered saline. The appendices were also immunohistochemically stained for CD4 and CD8 and the presence of CD4+ and CD8+ lymphocytes in the lavage fluid was determined by FACS analysis. Despite a macroscopically normal appearance, appendices of most UC patients showed histological characteristics of mucosal inflammation, with increased mucosal CD4+ lymphocytes. An increased CD4 proportion in appendiceal lavage fluid was predictive of a high appendiceal Geboes score in UC patients, and correlated with clinical and immunohistochemical findings in UC, CD and AA patients. In addition, IBD patients showed a distinct immunological profile with increased CD4+/CD8+ ratio.

Part II: Disease behaviour, quality of life and the risk of colectomy

Since the introduction of biologicals, limited studies have shown to what extent the disease course and colectomy rates have changed. Chapter 5 describes a retrospective cohort study of 506 UC patients in which proximal disease extension over time, disease behaviour patterns and risk factors for proximal disease extension and colectomy were evaluated. One third of UC patients with left-sided disease at diagnosis extended proximally during 10 years of follow up. The cumulative colectomy rate did not decrease over time. However, the median time until colectomy was longer than previously described (4,5 years versus approximately 2 years), which suggests that the advent of biologicals may not prevent

but rather delay colectomy in patients with more severe disease. Furthermore, proximal disease extension was not a risk factor for colectomy, but the risk of colectomy was rather determined by continuous disease activity, and use of systemic steroids and cyclosporine. Chapter 6 describes a cohort study in which health related quality of life (HRQL) and disability were compared in UC patients who underwent pouch surgery to patients who received anti-TNF treatment. Fifty-nine pouch patients were matched to 59 anti-TNF treated patients and showed that pouch patients had a significantly higher stool frequency, used more anti-diarrheal medication and had more peri-anal skin irritation problems. These findings did not influence overall disease specific disability outcomes. In fact, pouch patients showed better general health perspectives.

Part III: Restorative proctocolectomy and ileal pouch anal anastomosis

Restorative proctocolectomy with ileal pouch anal anastomosis is widely accepted as the standard procedure for patients with UC, indeterminate colitis, familial adenomatous polyposis (FAP) and selected cases of Crohn's disease. Anastomotic leakage represents a major early complication after IPAA surgery, which can lead to pouch dysfunction or pouch failure. In Chapter 7, risk factors associated with anastomotic leakage after restorative proctocolectomy with IPAA in a multicentre cohort of 640 patients were identified. Long-term disease course and the concurrent combination of steroid and anti-TNF treatment before IPAA surgery were independent risk factors for anastomotic leakage in IBD patients that underwent a proctocolectomy and IPAA. Being overweight and high ASA score were independent risk factors in patients that underwent a completion proctectomy and IPAA at a later stage. Identification of patients at high risk for anastomotic leakage is of utmost importance. These risk factors enable a tailored approach (staged procedure) in patients undergoing IPAA surgery. A defunctioning ileostomy, however, did not show a reduced leak rate, whereas a completion proctectomy and IPAA at a later stage reduced the leak rate by almost 50%. In Chapter 8, we further assessed this finding by looking into the short and long term outcome of selective ileostomy formation. This retrospective cohort study of 621 patients demonstrates that anastomotic leakage was high in all patients, even if the IPAA was defunctioned. Having steroids and anti-TNF had an even higher leak rate, which did not change by defunctioning the IPAA. Furthermore, primary ileostomy showed to be independently associated with long-term morbidity such as SBO, strictures and fistulas while no beneficial effect on the cumulative pouch failure rate were found.

The Cleveland Clinic has proposed a prognostic model of preoperative risk factors of pouch failure, which incorporated four predictive variables: completion proctectomy, handsewn anastomosis, diabetes mellitus and Crohn's disease. In Chapter 9, the Cleveland Clinic prognostic model was externally validated in a new cohort of 747 consecutive patients that underwent pouch surgery. In the study period, 45 patients had pouch failure, with a

median time to pouch failure of 31 months [IQR 9 - 82]. Multivariable analysis showed hand-sewn anastomosis to be the only significant independent predictor. The Harrell's concordance error rate was 0.42, indicating poor performance and therefore not suitable for application in daily clinical practice.

IBD in children is described to be more severe with more extensive inflammation, aggressive disease behaviour and eventually a higher risk of colectomy compared to adult-onset IBD. Studies comparing outcome of pouch surgery between children and adults are limited. Chapter 10 describes a cohort study in which adverse events and pouch function was evaluated between pediatric and adult patients who underwent IPAA. In total, 41 pediatric (median age 15 years) and 404 adult patients (median age 39 years) were included. Long-term pouch failure rates and pouch function were similar between pediatric and adult patients. Therefore, there is no need for a more cautious attitude in the application of IPAA in pediatric patients based on concerns of poor outcome.

Up to 50% of patients that underwent pouch surgery may develop pouchitis. A subgroup of these patients will also develop inflammation in the afferent ileum proximal to the pouch, a condition named pre-pouch ileitis, which is suggested to be a distinct disease entity. Chapter 11 assesses the incidence of pre-pouch ileitis, identified predictive factors and evaluated response to treatment in a cohort of 542 pouch patients.

Pre-pouch ileitis was diagnosed in 33 (6%) patients, 142 patients had pouchitis without pre-pouch inflammation. Although some patients (9/33) responded well to antibiotic treatment, the majority of patients (18/33) required steroids/immunomodulators and anti-TNF agents. In this sense, pre-pouch ileitis may be considered as a distinct clinical entity for which a different treatment algorithm is needed.

FUTURE PERSPECTIVES

This thesis sheds light on the peculiar association between the appendix and ulcerative colitis and has induced an exciting new research niche that has long been overlooked. As shown in Chapter 1 and 2, evidence is emerging suggesting an association between the appendix and UC; however, firm conclusions cannot yet be drawn. Currently, we are conducting the ACCURE trial in which UC patients are treated until remission is achieved and subsequently randomized to either undergo appendectomy or standard medical treatment. Endpoints of this study include the one-year cumulative UC relapse rate, the number of relapses per patient in the first year after randomisation, disease activity, health related quality of life and the number of colectomies. In the UK, a feasibility study (ACCURE-UK) has been conducted to explore if the appendectomy intervention is an acceptable treatment option to UC patients and clinicians and estimate the morbidity profile after surgery. The results of these studies will be awaited with great interest. Although the ACCURE study will give much clarity about the effect of an appendectomy in UC patients, some points should be stressed and taken in consideration in future studies.

In medicine, it is hardly the case where a "one fits all' approach could be practised. Specifically in IBD, we have come to an understanding that these multifactorial diseases encompass a broad spectrum of clinical phenotypes and ages of onset. The clinical presentation often differs depending on childhood or adult onset of the disease, with greater heterogeneity observed in adults. Therefore, neither with medication, nor surgery, will we be able to treat patients effectively if we do not incorporate baseline, disease and treatment factors. Chapter 3 has pointed us towards the idea that only a specific group of patients may benefit an appendectomy. If we could predict which patients would benefit an appendectomy, we would be able to perform more focused research. We demonstrated that appendiceal inflammation was highly predictive of pathological response when compared to no inflammation or extensive ulcerations. Therefore, futures studies should always incorporate pathological evaluation, specifically considering the growing interest in histological healing as a therapeutic goal in UC.24

In the ACCURE study, only patients that were in remission and exclusively treated with 5-ASA or immunomodulators were included. Obviously, we won't be able to extrapolate the results to patients with active disease or patients that were treated until remission with biologicals. Therefore, it would be interesting to include all UC patients, irrespective of disease activity in a future "ACCURE 2" study, in which we could stratify to disease activity and medication at a later stadium. Of course, this study design will need a much larger sample size for which more international collaborations are needed. International collaborations will enable us to perform more extensive research in a shorter amount of time, and consequently with less costs.

Considering whether the "ACCURE 2" should be a randomized controlled design is still a subject for debate. One of the pitfalls of a RCT is that only a small fraction of the original population is included, which is a biased group with regard to prognostic risk, thereby limiting generalizability. A well-designed observational study can produce similar results, even though a RCT rank first in the hierarchy of clinical evidence.25 In the ACCURE study, our experience was that patients decline participation due to their preference for an appendectomy. Furthermore, in surgical trials, blinding is not possible and may therefore bias assessment of outcomes. Whereas medical trials incorporate placebo medications to achieve blinding, surgical treatments may need a 'sham' procedure, which is not ethically approved. Nevertheless, placebo effect is inevitable, as shown in the most stringent RCT's, with double blinded study groups. A paper by Su et al., reviewed all placebo-controlled RCTs for active UC and found placebo remission rates to be as high as 40%. Factors that were associated with a lower placebo effect and should be considered in a future study design were short study duration, a low number of study visits, a stringent definition of outcome parameters and preferably including an objective measurement in the definition of remission such as endoscopic mucosal healing.26

Many questions regarding the immunological function and its role in the pathogenesis of UC remain currently unanswered. The contribution of microbiota in the pathophysiology of IBD is evident. Considering that the function of the appendix originally lies in the handling of commensal bacteria suggests that the appendix is involved in the development of UC. However, research evaluating the microbiome of the appendix in UC is lacking. Further research should focus upon identification of causal bacteria associated with UC and the diversity within the appendix. Novel data concerning the interaction between appendiceal microbiota and the deranged mucosal immune system may identify targets that play an immunomodulatory role in UC and give us a rationale for a therapeutic appendectomy.

The second and third part of this thesis demonstrates that surgery is inevitable in almost a quarter of all UC patients. Colectomy rates did not decrease over time, despite the introduction of biologicals. However, the median time until colectomy was longer than previously described which suggests that biologicals may not prevent but rather delay colectomy. It is questionable whether surgery should be delayed since there may be an increased risk for postoperative complications, specifically when treated with high doses of corticosteroids and biologicals (Chapter 8). Therefore, a multidisciplinary approach is warranted to decide the optimal timing for surgery. A restorative proctocolectomy with IPAA is nowadays the best surgical treatment option with satisfactory postoperative outcomes. As demonstrated in Chapter 6, patients that underwent pouch surgery reported better general health perspectives when compared to patients that were treated with biologicals, most likely due to the presumed 'curative' character of surgery. These findings provide applicable information to guide patients in clinical decision-making. Future prospective studies may be beneficial in order to obtain more insights with regard to quality of life and disability at different time points during the disease course.

To enable a tailored surgical strategy in patients undergoing IPAA surgery, reliable identification of patients at high risk for postoperative complications is of utmost importance. Risk stratification will influence surgical decision-making both in timing of the surgery and patient preparation. Although the preoperative risk factors, as demonstrated in Chapter 7, can be used in clinical decision-making, validated prediction models are lacking. Due to the retrospective nature of most studies, important variables could not be evaluated. Prospective controlled studies are necessary to gather a complete set of variables in order to develop and validate an accurate prediction tool.

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Nederlandse samenvatting

Nederlandse samenvatting

Colitis ulcerosa (CU) is een chronische inflammatoire darmziekte (IBD) dat veelal op jong volwassenen leeftijd ontstaat. Het ziekte beloop is onvoorspelbaar waarbij perioden van remissie zich afwisselen met exacerbaties. Hoewel de exacte etiologie nog niet geheel opgehelderd is, wordt verondersteld dat op basis van erfelijke gevoeligheid, een ongecontroleerde immuunreactie op commensale darmflora, mucosale ontsteking veroorzaakt. In de huidige behandelrichtlijn wordt een 'step-up' strategie gehandhaafd waarbij in de eerste instantie wordt gestart met betrekkelijk milde en relatief goedkope middelen zoals mesalazine en corticosteroïden. Bij geen of onvoldoende respons wordt overgegaan op immunomodulatoren en biologicals.

Ondanks grote vooruitgang in de medicamenteuze behandeling van CU, faalt een kwart van de patiënten waardoor een proctocolectomy met pouch noodzakelijk is.
Hoewel de appendix doorgaans werd beschouwd als een evolutionair overblijfsel, zijn er toenemend aanwijzingen dat er een relatie bestaat met het ontstaan en in stand houden van CU. Het onderzoek in dit proefschrift richt zich op de rol van de appendix in CU, het ziekte beloop van CU patiënten sinds de introductie van biologicals en de mogelijke risico factoren voor post operatieve complicaties na pouch chirurgie.

Deel I: het effect van een appendectomie in CU patiënten, experimentele en klinische studies
In **hoofdstuk 1** wordt een uitgebreide overzicht gegeven van de huidige literatuur betreffende klinische en immunologische aspecten van de appendix in IBD. Een systematische review met meta-analyse werd verricht waarbij in totaal 38 case controle studies werden geïncludeerd waarbij de beschermede rol van een appendectomie in IBD werd onderzocht. Merendeel van de studies toonden een beschermend effect na appendectomie in het ontwikkelen van CU met een odds ratio van 0.39 (95% CI 0.29 – 0.52). Drie van de 4 grote epidemiologische studies toonden een significant lagere CU incidentie ratio na een appendectomie. Zes observationele studies onderzochten het therapeutische effect van een appendectomie op het klinisch beloop van CU patiënten. Merendeel van de studies toonden minder opvlammingen, minder corticosteroïden gebruik en een lagere kans op colectomie na appendectomie vergeleken met patiënten die geen appendectomie waren ondergaan. Hoewel het exacte mechanisme nog onduidelijk is waarmee de relatie tussen de appendix en CU verklaard wordt, worden enkele theorieën gesuggereerd aan de hand van de literatuur. De invloed van de microbiota, mogelijk gehuisvest in de appendix, en 'homing' van bepaalde immuun populaties worden hierin verondersteld. **Hoofdstuk 2** gaat dieper in op de histologie, fysiologie en immunologische rol van de appendix in een gezonde en zieke mens. Het belang van de appendix als onderdeel van GALT (Gut Associated Lymphoid Tissue) en de biofilm worden beschreven. De biofilm is een slijmlaag met commensale

bacteriën dat wordt beschouwd als een belangrijke afweer mechanisme tegen pathogenen in de colon.

De vroege resultaten van de PASSION studie suggereert dat CU patiënten met mucosale appendicitis mogelijk profijt hebben van een appendectomie. In **hoofdstuk 3** werden therapie refractaire CU patiënten die verwezen waren voor een proctocolectomy gevraagd eerst een appendectomie te ondergaan. Klinisch effect, remissie, falen op appendectomie en pathologisch effect werden bepaald na 3 en 12 maanden. In totaal werden 30 patiënten geïncludeerd met een gemiddelde preoperatieve totale Mayo score van 9 (IQR 8 – 11). Na 3 maanden werd een pathologische effect in 11/22 patiënten gezien. Actieve ontsteking in de appendix was zeer voorspellend voor pathologisch effect in vergelijking met appendix preparaten zonder ontsteking of ernstige ulceraties. Na 12 maanden hadden 11/30 patiënten gefaald, 9 patiënten toonden klinisch effect na appendectomie waarvan 5 in remissie waren. Lange termijn data is echter noodzakelijk om een mogelijke placebo effect uit te sluiten. Als het immunologische fenotype van de appendix het klinisch effect op appendectomie zou voorspellen, kan dit gebruikt worden om patiënten vroegtijdig te identificeren die baat zouden kunnen hebben op een operatie. In **Hoofdstuk 4** laten we een interessant nieuw concept zien waarbij lymfocyten infiltratie werd bepaald in appendix lavage vloeistof. De appendix van 41 patiënten (15 CU, 9 ziekte van Crohn, 7 appendicitis acuta, 10 niet-inflammatoire controle patiënten) werden chirurgisch verwijderd en geflushed met 2ml fosfaat bufferde zout. De appendix preparaten werden immunohistologisch gekleurd op CD4 en CD8. In het lavage vloeistof werden CD4+ en CD8+ lymfocyten bepaald middels FACS analyse. Ondanks dat de appendices macroscopisch normaal waren, toonden de meeste appendix preparaten histologische eigenschappen van mucosale inflammatie met verhoogde CD4+ lymfocyten infiltratie. Een toegenomen CD4 proportie in het lavage vloeistof was voorspellend voor een hoge Geboes score in CU patiënten en correleerde met klinische en immunologische bevinden in CU, ziekte van Crohn en appendicitis acuta patiënten. Daarnaast, toonde IBD patiënten een duidelijk immunologisch profiel met een verhoogd CD4+/CD8+ ratio.

Deel II: Ziekte beloop, kwaliteit van leven en het risico op colectomie

Sinds de introductie van de biologicals, zijn er weinig studies gepubliceerd dat het ziekte beloop en risico op colectomie onderzoeken. **Hoofdstuk 5** beschrijft een retrospectieve cohort van 506 CU patiënten met proximale ziekte uitbreiding over een verloop van tijd, ziekte gedrag en het risico op ziekte uitbreiding en colectomie. Bij een derde van de patiënten met linkszijdige ziekte ten tijde van diagnose, werd een uitbreiding van ziekte naar proximaal gezien over een periode van 10 jaar. Het cumulatieve colectomie aantal bleef deze periode gelijk, hoewel de mediane tijd tot colectomie korter werd (4,5 jaar ten opzichte van 2 jaar). Deze bevinding zou kunnen betekenen dat biologicals de tijd tot colectomie eerder vertragen dan een operatie voorkomt. Het risico op een colectomie was

geassocieerd met continue ziekte activiteit, het gebruik van systemische corticosteroïden en ciclosporine. **Hoofdstuk 6** beschrijft een cohort studie waarin 'health related quality of life' (HRQL) en invaliditeit werden vergeleken tussen CU patiënten die een colectomie met pouch ondergingen en patiënten die werden behandeld met anti-TNF. In totaal werden 59 pouch patiënten gematched met 59 patiënten behandeld met anti-TNF waarbij we hebben aangetoond dat pouch patiënten een significant hoger ontlasting frequentie hadden, meer anti-diarree medicatie gebruikten en meer perianale huid irritatie hadden. Desondanks, hadden deze symptomen geen invloed op het totale ziekte-specifieke invaliditeitsuitkomsten met daarbij een betere algemene ziekte beleving.

Deel III: Proctocolectomie en ileo-anale pouch anastomose

Proctocolectomie met een ileo-anale pouch anastomose wordt wereldwijd beschouwd als de standaard ingreep bij patiënten met CU, indeterminate colitis, familiaire polyposis coli (FAP) en enkele streng geselecteerde patiënten met de ziekte van Crohn. Naadlekkkage is een gevreesde vroege complicatie na pouch chirurgie dat kan lijden tot pouchdysfunctie en pouchfalen. In **hoofdstuk 7** werden risico factoren voor naadlekkage na pouch chirurgie geïdentificeerd in een multicenter cohort van 640 patiënten. Een lange ziekte duur en gelijktijdig gebruik van corticosteroïden en anti-TNF voor pouch chirurgie waren onafhankelijke risico factoren voor naadlekkage. Overgewicht en een hoge ASA score waren onafhankelijke risico factoren voor naadlekkage in patiënten die een subtotale colectomie ondergingen met op een later moment pouch reconstructie. Het identificeren van patiënten die een hogere risico hebben op naadlekkage is belangrijk. Met behulp van deze risico factoren is het mogelijk om voor ieder patiënt die een pouch operatie moet ondergaan, een individuele behandel strategie te plannen. Een van de belangrijkste bevindingen was dat het aantal naadlekkages niet verminderde met een 'beschermende' ileostoma. Daarentegen, toonde het gefaseerd verrichten van een pouch operatie, waarbij eerst een subtotale colectomie werd verricht en op een later moment de pouch aangelegd een vermindering van het aantal lekkages met 50%. In **hoofdstuk 8** werden deze bevindingen verder onderzocht, waarbij de korte en lange termijn uitkomsten na het aanleggen van een ileostoma werden geanalyseerd. Deze retrospectieve cohort van 621 patiënten toonde een hoge naadlekkage percentage in zowel patiënten met een ileostoma als zonder. Corticosteroïd en anti-TNF gebruik verdubbelde zelfs het lekkage percentage, waarbij geen verschil werd gezien tussen patiënten met en zonder een ileostoma. Bovendien was een primaire ileostoma geassocieerd met lange termijn morbiditeit (ileus, stricturen en pouchfistels), zonder effect op het percentage pouchfalen. De Cleveland Clinic heeft een prognostische model voor pouchfalen samengesteld waarin 4 risico factoren zijn inbegrepen: restproctectomie, handgelegde anastomose, diabetes mellitus en de ziekte van Crohn. In **hoofdstuk 9** werd het prognostisch model van de Cleveland Clinic extern gevalideerd in een nieuwe cohort

van 747 patiënten die een pouch operatie waren ondergaan. Gedurende studie periode hadden 45 patiënten pouchfalen met een mediane tijd tot falen van 31 maanden [IQR 9 - 82]. Multivariabele analyse toonde een hand gelegde anastomose als enige onafhankelijke predictor voor pouchfalen. De Harrels concordance error rate was 0.42, wat duidde op matig functioneren van het model waardoor het niet geschikt is voor klinisch gebruik.

IBD op kinderleeftijd presenteert zich vaak agressiever met uitgebreide ziekte met een hogere kans op een colectomie in vergelijking met IBD op volwassen leeftijd. Er zijn weinig studies gepubliceerd die direct kinderen met volwassenen vergelijken die een pouch operatie ondergaan. **Hoofdstuk 10** beschrijft een cohort studie waarin morbiditeit en pouch functie wordt vergeleken tussen kinderen en volwassenen die een pouch operatie ondergaan. In totaal werden 41 patiënten (mediaan 15 jaar) en 404 volwassenen (mediaan 39 jaar) geïncludeerd. Het percentage pouchfalen en pouchfunctie was niet verschillend tussen kinderen en volwassenen. Hierdoor kan verondersteld worden dat een pouch operatie, een veilige behandeling is voor kinderen met dezelfde risico's als bij volwassenen.

Pouchitis kan in tot wel 50% van de patienten met een pouch ontstaan. Een subgroep van deze patiënten ontwikkeld ook ontsteking in het aanvoerende lis van de pouch dat doorgaans ook pre-pouch ileitis wordt genoemd. In **hoofdstuk 11** werden 542 pouch patienten geëvalueerd waarbij in 33 (6%) patiënten pre-pouch ileitis werd gezien en in 142 pouchitis zonder pre-pouch ileitis. Negen van de 33 patiënten respondeerde goed op antibiotica, echter had merendeel van de patiënten (18/33) een ophoging naar andere medicatie nodig zoals corticosteroïden, immunomodulatoren en anti-TNF. Pre-pouch ileitis moet hierdoor worden beschouwd als een apart entiteit waarvoor een ander behandelstrategie noodzakelijk is.

PhD portfolio

PhD Portfolio

General courses	Year	Workload
Basic Course Legislation and Organization for Clinical Researchers	2012	0.9
Clinical Data Management	2012	0.3
Advanced topics in epidemiology	2013	1.1
Infectious disease	2013	0.3
Reference manager	2013	0.6
Computing in R	2013	0.6
Biochemistry and molecular biology	2013	0.4
Scientific writing in English for publication	2013	1.5

Seminars, workshops and master classes	Year	Workload
Weekly department seminars Surgery	2012 - 2016	3.0
Weekly department seminars Gastroenterology and Hepatology	2012 - 2016	3.0
Post graduate course, United European Gastroenterology Week, Barcelona	2015	0.8

Oral presentations	Year	Workload
A multicenter evaluation of clinical and surgical risk factors for anastomotic leak after restorative proctocolectomy with ileal pouch-anal anastomosis		
Congress of European Crohn's and Colitis Organization, Kopenhagen	2014	0.5
Digestive Disease Week, Chicago	2014	0.5
Active inflammation of the appendix in ulcerative colitis		0.5
IBD today and tomorrow, AMC	2014	
Defunctioning ileostomy does not prevent anastomotic leaks after restorative proctocolectomy		0.5
Nederlandse Vereniging voor Gastroenterologie, Veldhoven	2014	0.5
Disease progression is a risk factor for colectomy in ulcerative colitis: 10-years of follow up in a tertiary care facility		
Nederlandse Vereniging voor Gastroenterologie, Veldhoven	2015	0.5
Incidence and severity of pre-pouch Ileitis: a distinct disease entity or a manifestation of refractory pouchitis?		
Nederlandse Vereniging voor Gastroenterologie, Veldhoven	2015	0.5
The role of the appendix in UC, an update on the clinical trials (masterclass)		
Congress of European Crohn's and Colitis Organization, Barcelona	2015	2.0
The PASSION study: compassionate use appendectomy for therapy refractory ulcerative colitis		
The United European Gastroenterology Week, Wenen	2016	0.5
Nederlandse Vereniging voor Gastroenterologie, Veldhoven	2016	0.5
International Colorectal Forum, Villars	2017	0.5
IBD in children: different outcomes? (masterclass)		
International EAES congress, Amsterdam	2016	2.0

Appendiceal lavage fluid discriminates between IBD and non-IBD patients		
The Japanese Society of Gastroenterology, Tokyo	2016	0.5

(Inter)national conferences	Year	Workload
Digestive Disease Week	2012 - 2016	5.0
The Japanese Society of Gastroenterology	2016	1.25
United European Gastroenterology Week	2012 - 2016	5.0
Congress of European Crohn's and Colitis Organization	2012 - 2016	5.0
NVGE voorjaarsdagen	2012 -2015	3.75
Chirurgendagen	2012 - 2015	3.75

Teaching	Year	Workload
Tutoring students in bachelor thesis		
Isabelle Kooij: Bachelor thesis (first prize bachelorthesis award)	2014	0.5
Sanne Uitentuis: Honneurs programme	2014	0.5
Kadere Konté: Scientific internship	2015	0.5
Alice Melle: Scientific internship	2015	0.5
Nathalie Versluis: Bachelor thesis	2016	0.5
Ellen Boon: Bachelor thesis	2016	0.5

Parameters of esteem	Year	Workload
Poster Champ Award & travelgrant, UEGW, Wenen	2014	
Best oral poster award, ECCO, Kopenhagen	2014	
The Young Investigators' Award & travelgrant, Japanese Society of Gastroenterology, Tokyo	2016	

Other	Year	Workload
Master Evidence Based Practice (MSc)	2014 -2015	97.0

List of publications

LIST OF PUBLICATIONS

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2. Colombo F, **Sahami S**, de Buck Van Overstraeten A, Tulchinsky H, Mege D, Dotan I, Foschi D, Leo CA, Warusavitarne J, D'Hoore A, Panis Y, Bemelman W, Sampietro GM. *Restorative Proctocolectomy in Elderly IBD Patients: A Multicentre Comparative Study on Safety and Efficacy.* J Crohns Colitis. 2016 Dec 7. pii: jjw209. doi: 10.1093/ecco-jcc/jjw209.

3. Van Gennep S, **Sahami S**, Buskens CJ, van den Brink GR, Ponsioen CY, D'Hoore A, de Buck van Overstraeten A, van Assche G, Ferrante M, Vermeire S, Bemelman WA, D'Haens GR, Löwenberg M. *Comparison of health-related quality of life and disability in ulcerative colitis patients following restorative proctocolectomy with ileal pouch-anal anastomosis versus anti-tumor necrosis factor therapy.* Eur J Gastroenterol Hepatol. 2017 Mar;29(3):338-344.

4. **Sahami S**, Bartels SAL, Young Fadok T, Tanis PJ, Wolthuis AD, de Buck van Overstraeten A, Buskens CJ, D'Hoore A, Bemelman WA. *External validation of a prognostic model of preoperative risk factors of failure of restorative proctocolectomy.* Colorectal Dis. 2016 Jun 18. doi: 10.1111/codi.13414.

5. Kooij IA, **Sahami S**, Meijer SL, Buskens CJ, Te Velde AA. *The immunology of the vermiform appendix: a review of the literature*. Clin Exp Immunol. 2016 Oct;186(1):1-9. doi: 10.1111/cei.12821. Review.

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7. **Sahami S**, Buskens CJ, Young Fadok T, Tanis PJ, de Buck van Overstraeten A, Wolthuis AD, Bemelman WA, D'Hoore A. *Defunctioning ileostomy is not associated with reduced leakage in proctocolectomy and ileal pouch anastomosis surgeries for IBD*. J Crohns Colitis. 2015 Oct 28.

8. **Sahami S**, Bartels SAL, D'Hoore A, Young Fadok T, Tanis PJ, Lindeboom R, de Buck van Overstraeten A, Wolthuis AD, Bemelman WA, Buskens CJ. *A multicentre evaluation of clinical and surgical risk factors for anastomotic leak after restorative proctocolectomy with ileal pouch-anal anastomosis for inflammatory bowel disease.* J Crohns Colitis. 2015 Sep 27.

9. **Sahami S**, Kooij IA, Meijer SL, Van den Brink GR, Buskens CJ, te Velde AA. *The Link between the Appendix and Ulcerative Colitis: Clinical Relevance and Potential Immunological Mechanisms.* Am J Gastroenterol. 2015 Sep 29.

10. Samaan MA, de Jong D, **Sahami S**, van den Brink GR, Löwenberg M, Ponsioen CY, Buskens CJ, Tanis PJ, de Buck van Overstraeten A, D'Hoore A, Bemelman WA, D'Haens GR. *Incidence and Severity of Prepouch Ileitis: A Distinct Disease Entity or a Manifestation of Refractory Pouchitis?* Inflamm Bowel Dis. 2015 Sep 16.

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14. Buskens CJ, **Sahami S**, Tanis PJ, Bemelman WA. *The potential benefits and disadvantages of laparoscopic surgery for ulcerative colitis: A review of current evidence.* Best Pract Res Clin Gastroenterol. 2014 Feb;28(1):19-27. doi: 10.1016/j.bpg.2013.11.007. Epub 2013 Dec 4. Review.

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About the author

ABOUT THE AUTHOR

Saloomeh Sahami was born on December 5th, 1983 in Mashhad, Iran. At the age of 4, she and her family moved to the Netherlands. She went to high-school at college Hageveld in Heemstede and graduated medical school at the University of Amsterdam.

She wrote her thesis about the role of the appendix in ulcerative colitis, the disease course of ulcerative colitis patients and postoperative complications after pouch surgery at the department of Gastroenterology and Hepatology and department of Surgery.

Her enthusiasm for research was founded during her medical studies in 2007, where she performed field research at the department of Cardiology. She gathered and evaluated data from automated external defibrillators for the ARREST study, a large multicenter study for prehospital recognition and management of patients with chest pain. In 2011, she trained to become a clinical epidemiologist and obtained her masters degree in Evidence Based Practice at the University of Amsterdam in 2013.

After graduating medical school in July 2012, she started her PhD training under supervision of prof. dr. Willem Bemelman and prof. dr. Geert D'Haens focusing on both clinical and experimental studies in ulcerative colitis patients. Her daily supervisor was dr. Christianne Buskens, and experimental studies were supervised by prof. dr. Gijs van den Brink.

During her clinical rotations and PhD training she became interested in Gastroenterology and Hepatology. Since January 2016, Saloomeh has worked at the department of Internal Medicine at the Alrijne hospital in Leiderdorp and started her internal medicine residency training in January 2017. In January 2019, she will start her Gastroenterology training at the Leiden University Medical Center (dr. A.M.J. Langers and dr. R.A. Veenendaal).

Stellingen

Behorende bij het proefschrift

NEW CONCEPTS IN ULCERATIVE COLITIS
A Thin Line Between Medicine And Surgery

1. An essential part of the immunological function of the vermiform appendix lies in the interaction with gut flora *(this thesis)*

2. Ulcerative colitis patients with mucosal appendicitis may benefit from appendectomy *(this thesis)*

3. One third of the patients with UC have inflammation that extends more proximally over time, which does not increase the risk for colectomy *(this thesis)*

4. The presumed 'curative' character of surgery in UC patients who underwent pouch surgery results in better general health perspectives compared to anti-TNF treated patients *(this thesis)*

5. A modified two-stage pouch procedure is the best strategy in patients treated with steroids and/or anti TNF *(this thesis)*

6. Handsewn anastomosis is associated with pouch failure and should therefore be discussed with the patient *(this thesis)*

7. There is no need for a more cautious attitude in the application of a pouch in children based on concerns of poor outcome *(this thesis)*

8. That the vermiform appendage of the caecum is a rudiment, we may infer from its small size and its variability in man *(Charles Darwin)*

9. Maybe you are searching among the branches, for what only appears in the roots *(Rumi)*

10. Adapt what is useful, reject what is useless and add what is specifically your own *(Bruce Lee)*

11. Everything not saved will be lost *(Nintendo "Quit Screen" message)*

12. Geen enkele wijze van planning, vervangt stom geluk

Saloomeh Sahami
2 Juni 2017