Development of pouchitis following ileal pouch-anal anastomosis (IPAA) for ulcerative colitis: A role for serological markers and microbial pattern recognition receptor genes

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Abstract

Background and Aims: Pouchitis, the most common complication after proctocolectomy with ileal pouch-anal anastomosis (IPAA) for ulcerative colitis, has been attributed to altered composition of faecal flora. We investigated the role of antimicrobial and antiglycan antibodies and polymorphisms in microbial pattern recognition receptor genes.

Methods: Clinical charts of all 184 patients with ulcerative colitis who underwent IPAA between 1990–2004 were reviewed for pre- and post-operative disease course.

Results: Follow-up data were available in 172 patients [67 female, median age at proctocolectomy 39.1 years]. During a median follow-up of 6.7 (interquartile range 3.7–10.5) years, 80 patients (47%) developed at least one episode of pouchitis. Cox proportional-hazard regression identified extra-intestinal manifestations [HR 1.78 (95%CI 1.10–2.88), p=0.020], a
Development of pouchitis following IPAA for UC

1. Introduction

Up to 30% of patients suffering from ulcerative colitis (UC) will ultimately need to undergo a total colectomy.¹ The most frequent indications for colectomy include intractable disease and the occurrence of dysplasia or cancer in case of long-standing colitis. A total proctocolectomy with ileal pouch-anal anastomosis (IPAA) has become the surgery of choice for the "definitive" management of UC, since it avoids a permanent stoma while removing all diseased colonic mucosa.²

Although the mortality rate after IPAA is acceptable (less than 1%),²,³ short term morbidity is seen in approximately 30% of patients, including post-operative bleeding, pouch leakage, pelvic abscess and fistula.²,⁴ On the long term, patients with a pouch can also be troubled by small bowel obstruction, faecal incontinence, sexual dysfunction and infertility.²,⁵ Pouch failure is seen in approximately 5% of patients.²,³,⁹

The most frequent long-term complication is the occurrence of pouchitis,¹⁰ with cumulative incidence rates varying significantly between studies (7 to 59%). Besides differences in length and type of follow-up, an important reason for this variation is a lack of universally accepted diagnostic criteria for pouchitis.¹¹ From a clinical perspective, different types of pouchitis exist.¹² Patients can present with one single episode of acute pouchitis, while others have acute relapsing pouchitis or a chronic unremitting course. In our own experience, 25% of patients developed pouchitis (proven by endoscopy and histology) within the first year after closure of ileostomy.⁹ During a median follow-up of 6.5 years, 46% of patient developed pouchitis, and more than half of them suffered from acute relapsing on chronic pouchitis.⁹

The aetiology of pouchitis is not entirely understood. Bacterial overgrowth, altered balance of luminal bacteria, mucosal ischemia, nutritional deficiencies, lack of short-chain fatty acids and faecal bile acids toxicity have all been suggested as possible etiological factors.¹¹ Factors that support the role of the microbial flora, are the fact that pouchitis is only seen after restoration of the faecal stream and can be treated successfully by use of antibiotics.¹¹,¹²

Bearing in mind the high cumulative incidence rate of pouchitis, several investigators have tried to define predictive factors which could help in selecting these patients in need for more intensive post-operative follow-up or post-operative prophylaxis. Established risk factors are non-smoking,¹³,¹⁴ and the presence of extra-intestinal manifestations, especially primary sclerosing cholangitis.⁹,¹⁴,¹⁵⁻¹⁷ Possible risk factors might be the extent of colitis and presence of backwash ileitis prior to surgery,¹⁷,¹⁸ a young age at diagnosis or surgery,⁹,¹⁴ dysplasia as indication for surgery,¹³,¹⁴ pre-operative use of steroids¹⁹ and the regular use of NSAIDs in the postoperative phase.¹³,¹⁷ More recently, an association was found between pouchitis and variations in the interleukin 1 receptor antagonist (IL-1RA) and tumor necrosis factor (TNF) genes.²⁰,²¹ Finally, pre-operative pANCA levels seem to be higher in patients who develop chronic pouchitis compared to patients who do not.²²

The primary aim of our study was to look for factors associated with pouchitis in patients who underwent a restorative proctocolectomy with IPAA for ulcerative colitis or IBD type unclassified (IBDU). Given presumed role of bacteria as main trigger in the onset of pouchitis, we were especially interested in the role of antimicrobial and antiglycan antibodies and polymorphisms in microbial pattern recognition receptor genes.

2. Material and methods

2.1. Patients

All patients who underwent a proctocolectomy with IPAA for ulcerative colitis (UC) or IBD type unclassified (IBDU) at the University Hospital Gasthuisberg in Leuven (tertiary referral centre) between January 1990 and December 2004 were identified through the surgical database. A total of 184 consecutive patients [113 male/71 female; median (inter-quartile range, IQR) age at diagnosis 29.5 (23.8–38.8) years; median (IQR) age at proctocolectomy 39.0 (30.4–47.7) years] were identified. However, we were unable to trace 11 out of 184 patients (5%) who had moved house without leaving updated contact details. The ileostomy could be closed in all but one patient. This patient was excluded for further analysis.

Clinical charts of all remaining 172 patients were reviewed to trace clinical, endoscopical and histological characteristics, including gender, age at diagnosis, age at proctocolectomy, age at closure of ileostomy, duration of disease prior to proctocolectomy, duration of follow-up after closure of ileostomy, diagnosis prior to proctocolectomy, diagnosis after proctocolectomy, familial history, extra-intestinal manifestations, smoking behaviour, therapy at proctocolectomy, extent of disease prior to proctocolectomy, presence of backwash ileitis. Surgical characteristics included type of surgery (laparoscopic vs. laparotomy), type of anastomosis (stapled without mucosectomy vs. handsewn with mucosectomy), type of pouch (J-pouch or S-pouch), number of stages (1-, 2- or 3-stage surgery), construction of the pouch at time of proctocolectomy. The main clinical and surgical characteristics are enlisted in Table 1.

The clinical charts were also reviewed to trace the occurrence of first pouchitis and the occurrence of chronic
pouchitis. In case of absence of follow-up at our tertiary referral centre in the last six months, we contacted the clinician (general practitioner or local gastroenterologist) following the patient.

Over the study period of 15 years, ileal pouch-anal surgery was performed by 4 experienced abdominal surgeons. Restorative proctocolectomy with IPAA was defined as a one-, two- or three-stage procedure. Importantly, the number of stages did not follow strict guidelines, but the surgical judgement was based on severity of colitis at the time of surgery and clinical condition of the patient. In general, pouch construction was not performed in the presence of risk factors that were identified to be related to or more than 200 mg hydrocortisone over 24 h. 23

Follow-up data were obtained by standardized and regular outpatient visits: one early postoperative visit six weeks after closure of ileostomy, followed by 3-monthly visits up to one year, and yearly visits thereafter, unless patients developed symptoms necessitating earlier visits. Follow-up visits included detailed history and clinical examination. The diagnosis of pouchitis was only made after confirmation on endoscopy and histological examination. If the clinician suspected pouchitis, an endoscopy was performed and biopsies were taken for histological examination. The diagnosis of pouchitis was only made after the patient.

Follow-up visits included detailed history and clinical examination. The diagnosis of pouchitis was only made after confirmation on endoscopy and histological examination. If the clinician suspected pouchitis, an endoscopy was performed and biopsies were taken for histological examination. The diagnosis of pouchitis was only made after confirmation on endoscopy and histological examination.

We performed a robust search of both the literature and the NCBI public database (<www.ncbi.nlm.nih.gov>) to reveal non-
synonymous single nucleotide polymorphisms (SNPs) in genes encoding for Toll-like receptors (TLR) 1 to 10. Besides 50 SNPs in TLR1-10, we also investigated previous reported SNPs in caspase recruitment domain CARD15, cluster of differentiation 14 (CD14) and TNF, as well as a variable number tandem repeat in IL-1RA. All SNPs were analyzed using PCR-RFLP.

In a cohort of 192 healthy controls, 37 SNPs did not exceed a prevalence of 1%. The remaining 19 polymorphisms were analyzed in 144 patients. The remaining 28 patients refused the extra blood withdrawal. Blood for genetic analysis was taken at time of informed consent and enrolment. Primers and restriction enzymes are available upon request (Supplementary File 1).

### 2.3. Serological analyses

Sera of 145 patients, obtained at time of informed consent and enrolment, were analyzed for expression of IgG anti-Saccharomyces cerevisiae (gASCA), IgG anti-laminaribioside (ALCA), IgA anti-chitobioside (ACCA), IgG anti-mannobioside (AMCA) and anti-outer membrane porin (Omp) antibodies in a blinded fashion, by one experienced lab technician. All assays were performed in our own laboratory by using ELISA as described by the manufacturers, Glycominds Ltd (Lod, Israel) and INOVA Diagnostics Inc (San Diego, CA, USA). Cut-off values for positivity were defined at 50 Elisa Units (EU) for

### Table 2 Results of genetic analyses (n=144)

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HWE: Hardy Weinberg Equilibrium.

### Table 3 Results of serological analyses (n=145)

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<td>Omp</td>
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<td>57/145 (39)</td>
<td>(25 EU)</td>
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<td>pANCA</td>
<td>65/145 (45)</td>
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IQR: interquartile range; EU: Elisa Units.
gASCA, 70 EU for ALCA, 90 EU for both ACCA and AMCA, and 25 EU for Omp.\textsuperscript{27} Perinuclear anti-neutrophil cytoplasmatic antibodies (pANCA) were determined by indirect immunofluorescence (IIF) using ethanol-fixed neutrophil slides (INOVA Diagnostics)\textsuperscript{27} with a cut-off value for positivity set at 1/40.

Figure 1 Pouchitis-free survival after closure of ileostomy (A), stratified by extra-intestinal manifestations (B), ACCA antibodies (C), TLR1 S87I genotype (D) and TLR6 S249P genotype (E).
2.4. Definitions

The diagnosis of pouchitis was based on typical clinical symptoms, in combination with a suggestive endoscopic image of inflammation of the pouch, confirmed by histology. Symptoms include watery, sometimes bloody diarrhoea, abdominal cramps, faecal urgency and tenesmus, general malaise and fever. Typical endoscopy findings are characterized by hyperaemic and/or hemorrhagic friable and granular mucosa with excessive mucopurulent areas and

![Figure 2](https://academic.oup.com/ecco-jcc/article-abstract/2/2/142/381950)

**Figure 2** Chronic pouchitis-free survival after closure of ileostomy (A), stratified by extra-intestinal manifestations (B), backwash ileitis (C), ACCA antibodies (D), Omp antibodies (E) and TLR2 R753Q genotype (F).
superficial erosions. Histological examination shows acute inflammation including neutrophilic infiltration and mucosal ulceration in addition to chronic inflammation, including villous atrophy, crypt hyperplasia, and a chronic inflammatory cell infiltration.11,12,28 Pouchitis was further categorized as acute, acute relapsing or chronic, as described previously.12 Pouchitis was considered acute if symptoms responded rapidly to medication and if the duration was less than 4 weeks. Starting from 3 or more acute episodes, pouchitis was defined acute relapsing. Pouchitis was considered chronic if symptoms lasted for more than four weeks, despite standard therapy.12

2.5. Statistical analysis

The cumulative incidence of pouchitis and chronic pouchitis was estimated by means of Kaplan–Meier analyses. We used both Breslow and LogRank tests to compare hazard rates in populations defined by one variate at the time. Since the length of follow-up varied significantly between patients, the most appropriate test to look for predictors is the Breslow test, also known as Gehan Wilcoxon test, where time points are weighted by the number of cases at risk at each time point. However, we also included the most commonly used LogRank test, were all time point are weighted equally. This test has optimal power to detect alternatives where the hazard rates in the populations are proportional to each other.

A Cox proportional hazards survival regression including all variables with Breslow p<0.100, was performed to identify independent predictors. We checked departures from the proportional hazards model by introducing time-dependent explanatory variables, using log(t) and adjusted by the average of the logs (0.5). There was no indication that the proportionality of hazard assumption was violated. Since blood samples were only available in 144 out of 172 patients, Cox proportional hazards survival regression was only performed in this subgroup of patients. However, as shown in Table 1, the main characteristics of this subgroup did not differ from the overall cohort.

Other performed analyses included Chi Square statistics, Fisher’s Exact and Mann–Whitney U tests.

All statistical analyses were performed using the SPSS 15.0 (SPSS Inc, Chicago, IL, USA) and SAS 9.1 statistical software packages (SAS Institute Inc., Cary, NC, USA). The threshold for statistical significance was predefined as p<0.05.

3. Results

3.1. Demographics

Demographic and clinical characteristics of the 172 patients [67 female/105 male; median (IQR) age at diagnosis 31.3 (23.8–39.0) years; median (IQR) age at proctocolectomy 39.1 (30.8–47.8) years] are summarized in Table 1. These characteristics did not differ significantly in the subgroup of 144 patients who consented for both genetic and serological blood analyses (Table 1).

Prior to proctocolectomy, ulcerative colitis was diagnosed in 94% of the patients, based on clinical, radiological, endoscopical and histological findings.29 The remaining 11 patients were classified as inflammatory bowel disease type unclassified (IBDU).30 In 9 of these patients the diagnosis of IBDU could be changed to ulcerative colitis after thorough examination of the resection specimen and previous biopsies. Postoperatively, two patients were diagnosed with indeterminate colitis (IC). None of the patients who underwent a protocolectomy with IPAA had a pre- or peri-operative diagnosis of Crohn’s disease.

3.2. IPAA surgery and follow-up

Surgical characteristics are summarized in Table 1. Twenty-six patients (15%) underwent a one-stage procedure, while

![Figure 3](https://academic.oup.com/ecco-jcc/article-abstract/2/2/142/381950/148/M.Ferrante-et-al)
Development of pouchitis following IPAA for UC

thirty-one patients (18%) underwent a three-stage procedure (subtotal colectomy first, followed by completion proctocolectomy and IPAA, and finally ileostomy closure). The remaining 115 patients (67%) underwent a two-stage procedure. In 101 of these, the pouch construction was performed at the time of proctocolectomy (first stage), while in the remaining 14 patients pouch construction was performed after prior subtotal colectomy. The median (IQR) duration between proctocolectomy and closure of ileostomy was 11.7 (7.9–19.0) weeks.

3.3. Genetic and serological analyses

Genetic and serological analyses were performed in 144 and 145 patients, respectively. All but one SNP were in Hardy–Weinberg equilibrium. The main results are highlighted in Tables 2 and 3.

3.4. Pouchitis

During a median (IQR) follow-up of 6.7 (3.7–10.5) years, 80 patients (47%) developed at least one episode of pouchitis (Fig. 1A). The prevalence of pouchitis steadily increased over time, with cumulative incidence rates of 25%, 32%, 36%, 40% and 45% at 1, 2, 3, 4 and 5 years, respectively. Of all patients developing pouchitis, 32 (40%) developed less than three episodes of pouchitis during follow-up, 15 (19%) had acute relapsing pouchitis and 33 (41%) developed chronic pouchitis (Fig. 2A).

A Kaplan–Meier analysis revealed that patient with EIM prior to proctocolectomy (Breslow $p=0.006$, LogRank $p=0.011$), and positive ACCA antibodies (Breslow $p=0.041$, LogRank $p=0.040$) developed their first pouchitis episode more rapidly (Fig. 1B–C). Furthermore, patients with a GT/TT genotype at TLR1 S87I (Breslow $p=0.065$, LogRank $p=0.051$), and a CC/CT genotype at TLR6 S249P (Breslow $p=0.070$, LogRank $p=0.113$) showed a tendency to develop their first pouchitis more rapidly (Fig. 1D–E). Patients who developed pouchitis were significantly younger, both at diagnosis (median (IQR) age 27.81 (21.37–34.76) vs. 33.61 (24.67–43.21) years, $p=0.005$) and at proctocolectomy (median (IQR) age 35.50 (28.11–43.72) vs. 41.59 (33.84–49.89) years, $p=0.003$). Duration of disease prior to proctocolectomy was similar in both cohorts.

The Cox proportional hazard survival regression, identified presence of EIM [Hazard ratio 1.78 (1.10–2.88), $p=0.020$], presence of a GT/TT genotype at TLR1 S87I [Hazard ratio 1.64 (1.01–2.66), $p=0.047$], ACCA antibodies [Hazard ratio 2.03 (1.11–3.70), $p=0.021$] and a young age at diagnosis [Hazard ratio 0.99 (0.93–1.05), $p=0.003$] as independent factors associated with pouchitis. As Fig. 3A shows, the pouchitis risk significantly increased if more risk factors (EIM, backwash ileitis, ACCA antibodies and microbial pattern recognition receptor genes) were present (Breslow $p=0.001$, LogRank $p=0.001$).

In univariate analysis, chronic pouchitis was more rapidly diagnosed in patients with extra-intestinal manifestations (Breslow $p=0.004$, LogRank $p=0.010$), backwash ileitis (Breslow $p=0.001$, LogRank $p<0.001$), positive ACCA antibodies (Breslow 0.048, LogRank 0.091), positive Omp antibodies (Breslow $p=0.020$, LogRank 0.024), and a GA/AA genotype at TLR2 R753Q (Breslow $p=0.009$, LogRank $p=0.040$) (Fig. 2B–F).

Patients with pre-operative use of aminosalicylates tended to develop chronic pouchitis more rapidly (Breslow $p=0.082$, LogRank $p=0.102$). Patients who developed chronic pouchitis were significantly younger at proctocolectomy [median (IQR) age 35.24 (28.64–44.12) vs. 39.77 (31.16–49.23) years, $p=0.040$]. Age at diagnosis and duration of disease prior to proctocolectomy was similar in patients who developed chronic pouchitis and patients who did not.

The Cox proportional hazard survival regression, identified presence of EIM [Hazard ratio 2.45 (1.07–5.62), $p=0.034$], presence of backwash ileitis [Hazard ratio 3.15 (1.10–9.00), $p=0.032$], Omp antibodies [Hazard ratio 2.67 (1.20–5.94), $p=0.016$] and a young age at proctocolectomy [Hazard ratio 0.008 as independent factors associated with chronic pouchitis. As Fig. 3B shows, the chronic pouchitis risk significantly increased if more risk factors (EIM, backwash ileitis and Omp antibodies) were present (Breslow $p=0.001$, LogRank $p=0.002$).

4. Discussion

Pouchitis after proctocolectomy with ileal pouch-anal anastomosis for ulcerative colitis is still a major challenge. There is increasing evidence for a pathophysiological role of the microbial flora in the development of pouchitis. We looked for factors associated with pouchitis in 172 patients who underwent a restorative proctocolectomy with IPAA for UC or IBDU, and were especially interested in the role of antimicrobial and antiglycan antibodies and microbial pattern recognition receptor genes.

During a median (IQR) follow-up of 6.7 (3.7–10.5) years, 80 patients (47%) developed at least one episode of pouchitis. A Cox proportional hazard survival regression identified presence of EIM, a GT/TT genotype at TLR1 S87I, ACCA antibodies and a young age at diagnosis as independent factors associated with pouchitis. During the same follow-up period, 33 patients (19%) developed chronic pouchitis. Independent factors associated with chronic pouchitis, were presence of EIM, backwash ileitis, Omp antibodies and a young age at proctocolectomy.

The main benefits of this trial are the extended follow-up (median 6.7 years), the availability of longitudinal data and the separate analysis of patients with chronic pouchitis. In contrast to other investigators we did not analyze the subgroup of patients with acute and acute relapsing pouchitis separately, since these patients might still develop chronic pouchitis later on.

After Cox proportional hazard survival regression, we identified a GT/TT genotype at TLR1 S87I to be associated with the development of pouchitis. To our knowledge, this is the first time that a pattern recognition receptor gene has been implicated in the development of pouchitis. Interestingly, TLR1 is a co-factor of the TLR2 lipopolysaccharide receptor complex, supporting the hypothesis that bacterial recognition is important in the onset of pouchitis. We were not able to confirm the previously reported associations with a polymorphism in the promoter region of TNF neither with the IL-1RA gene. Like Lammers et al we did not find an association with the three common CARD15 mutations.

Since we were limited to SNPs which could be analyzed through PCR-RFLP, it is still possible that other pattern recognition receptor genes are associated with pouchitis.
Furthermore, we found that ACCA and Omp antibodies were independent factors associated with pouchitis and chronic pouchitis, respectively. This finding again supports the pathophysiological role of the microbial flora. The previously reported association between high pANCA levels and development of chronic pouchitis2,31 was not confirmed in our cohort. However, we did not use a quantitative ELISA method. The remaining anti-glycan antibodies ALCA, AMCA and gASCA were also not associated with the development of pouchitis. The lack of association with ASCA has also been demonstrated by other investigators.21,22,33,34 Recently, Fleshner et al reported an association with duration of disease.13

Concerning clinical factors, the presence of extra-intestinal manifestations was associated with both development of pouchitis and chronic pouchitis. Similar results have been found by several other investigators.14,17,19,35,36 The previously reported strong association with primary sclerosing cholangitis in particular could not be confirmed in our cohort, most likely due to the small number of patients with this extra-intestinal manifestation (n=4). Interestingly, 3 out of 4 patients with primary sclerosing cholangitis developed pouchitis within 2 years after closure of ileostomy. The role of backwash ileitis might also have been suggested in the past.18,37 However, while Schmidt et al and Abdelrazeq et al reported a higher frequency of pouchitis in patients with terminal ileal inflammation, we only demonstrated an association with chronic pouchitis. Both extra-intestinal manifestations and backwash ileitis might be associated with a bacterial overload. Experiments in animal models showed a failure to develop joint disease in HLA-B27 transgenic rats which were reared in germ-free conditions38 and an attenuation of the severity of joint disease was seen after treatment with metronidazole.39

Finally, we found a role for young age at diagnosis and young age at proctocolectomy in pouchitis and chronic pouchitis, respectively. Similarly, Stalhberg et al reported that patients who developed pouchitis were significantly younger at diagnosis of UC compared to patients who did not develop pouchitis.14 Like other investigators, we did not observe an association with duration of disease.13

The main limitations of our study are its retrospective character and the fact that this is not a gene-tagged approached. Furthermore, antibody responses towards Pseudomonas fluorescens-associated sequence 12 (anti-12)40 and flagellin CBir1 (anti-CBir1)41 were not evaluated, since these kits are not commercially available yet. Finally, most of the blood samples were not taken prior to proctocolectomy, which might have influenced the results of the serological markers. Although investigators always considered serological response stable over time, our group recently showed that the antibody response towards gASCA, ACCA, AMCA and Omp increased over time in a cohort of patients with Crohn's disease (CD).27 Data on the stability of markers in patients with UC are not available at the moment.

In conclusion, we demonstrated an association between serological markers (ACCA and Omp), a non-synonymous SNP in TLR1 and the development of pouchitis. Together with the effective use of antibiotics for the treatment of pouchitis and the observation that pouchitis only develops after restoration of the faecal stream, our results suggest a major pathophysiological role of the microbial flora. Further prospective trials are necessary to confirm our findings.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.crohns.2007.10.003.

References


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