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Review

The aetiology of pouchitis in patients with inflammatory bowel disease

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***Abstract*:** Restorative proctocolectomy with ileal pouch-anal anastomosis is a treatment option for patients with refractory ulcerative colitis. Pouchitis is the most common complication, representing a spectrum of diseases ranging from acute antibiotic-responsive type to chronic antibiotic-refractory. Early accurate diagnosis using a combined assessment of symptoms, endoscopy and histology is important for both treatment and prognostication. Most patients respond well to antibiotic therapy; however, management of chronic antibiotic-refractory pouchitis remains a challenge, and treatment options are based on small studies. Pouchitis is thought to be driven by the interaction between genetics, the immune system and the environment but as yet a causal relationship has yet to be identified. Further longitudinal assessment of the pouch integrating new technologies may help us understand the factors driving pouchitis. This review outlines the currently understood risk factors and aetiology of pouchitis.

Keywords: IBD, pouchitis, ulcerative colitis

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Introduction

Restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) is offered to patients with refractory ulcerative colitis (UC) as well as for some patients with familial adenomatous polyposis (FAP). Restorative proctocolectomy is typically associated with good functional outcomes; however, complications may arise in a proportion of patients. The most common complication of IPAA is pouchitis, which is inflammation of the pouch reservoir. Pouchitis can present with pelvic pain, increased stool frequency, urgency and hematochezia.

Currently, the diagnosis of acute pouchitis requires a combination of clinical, endoscopic and histological markers. The heterogeneous disease spectrum as well as the lack of classic pathognomonic features has made it difficult to develop a validated scoring system for pouchitis. Furthermore, the aetiology of pouchitis is poorly understood; though it is likely to be multifactorial in nature and involves a complex interplay between the host immune system and the gut microbiome.

Although no validated scoring system exists, the pouch disease activity index is currently the most widely adopted. It consists of three domains (clinical, endoscopic and histologic) corresponding to the findings of active inflammation of the pouch in a patient with compatible clinical presentation.[1] The diagnosis is further supported by non-invasive biomarkers such as faecal calprotectin and imaging studies such as magnetic resonance imaging, computed tomography (CT) and intestinal ultrasound.[2]

Pouchitis can be classified based on the duration of symptoms. Acute pouchitis occurs when clinical symptoms last for 4 weeks or less, whereas chronic pouchitis is usually defined by symptoms that last for more than 4 weeks.[1] Chronic pouchitis is further classified as either antibiotic-responsive or antibiotic-dependent, requiring ongoing antibiotic therapy to maintain

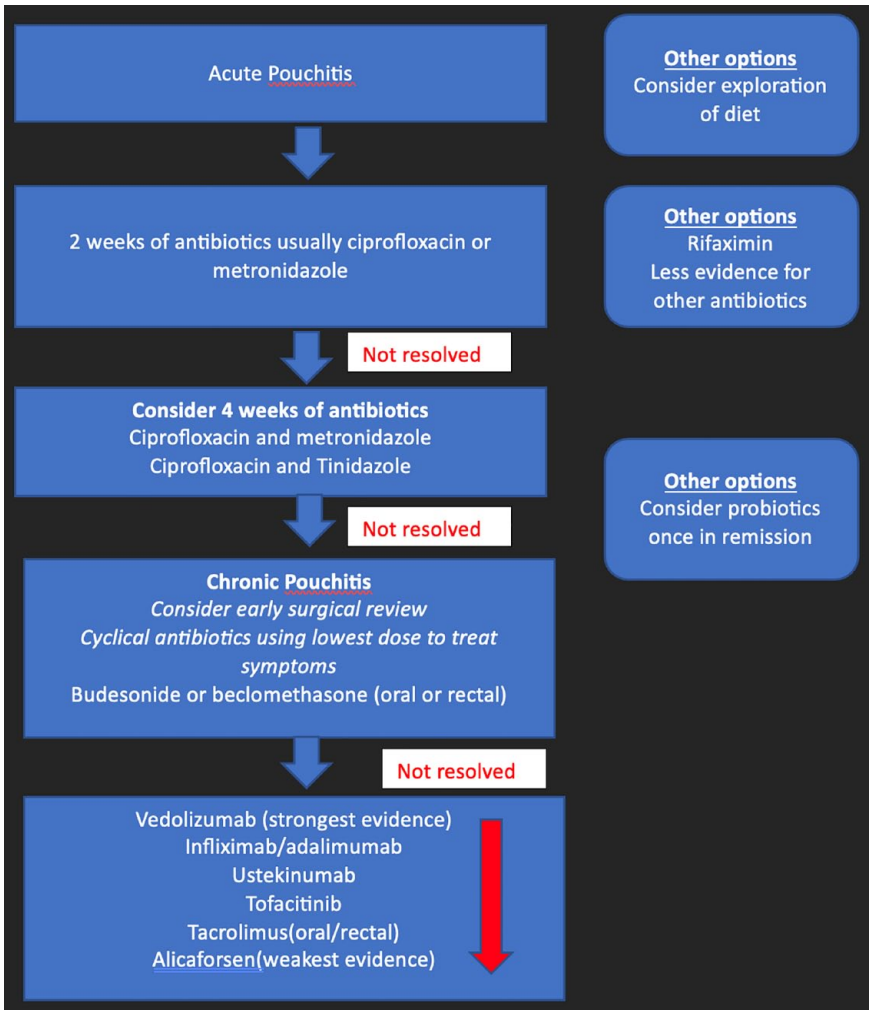

Figure 1. A stepwise approach to the management of both acute and chronic pouchitis. Acute pouchitis is typically managed with antibiotics. A prolonged course of symptoms despite antibiotics suggests chronic pouchitis, which may require treatment with steroids or a biological agent.

remission.[3] Antibiotic-refractory refers to cases not responding to ⩾4 weeks of therapy.[3] Most patients with acute pouchitis have an initial symptomatic resolution with a course of antibiotics. However, approximately 50–90% of patients will have at least one recurrence, of which 10–30% will eventually progress to chronic pouchitis.[4]

The risk of pouchitis development increases with time, with cumulative incidence rates of 25% at 1 year, 35% at 3 years and 45% at 5 years.[5,6] In a recent meta-analysis, the prevalence of pouchitis was found to be higher in patients with UC (32%) than patients with FAP (6%) with an odds ratio of 4.95 ($p < 0.0001$).[7]

This review will explore the current understanding of pouchitis aetiology as well as the risk factors associated with its development. Treatment of pouchitis has been explored previously and as such will not be covered in this review.[8] A figure summarizing a basic treatment algorithm for pouchitis is attached for reference (see Figure 1).

Pre-pouch risk factors for pouchitis

In understanding the aetiology of pouchitis, it is important to recognize factors associated with pouchitis prior to pouch formation. These include the type and severity of pre-existing bowel pathology, genetics, environmental and surgical factors.

The following sections will explore these factors individually.

Pre-existing bowel pathology

This is exemplified by the differences observed between pouches with FAP *versus* UC. Specifically, whilst pouchitis occurs in about 58% of patients with UC, it is only seen in about 5% of patients with FAP.[9] Given both UC and FAP pouch patients undergo the same surgical procedure, this suggests that the underlying pathology of UC may contribute to the development of pouchitis.[10] However, the fact that pouchitis does occur (albeit much less frequently) in FAP patients suggests that it is not simply a recurrence of the UC disease within the pouch, but rather a distinct process. Ultimately, the reason for this difference is not well understood, with both genetic susceptibility and pro-inflammatory cytokines thought to be key factors involved.

A recent hypothesis suggests an association between peripouch fat and a higher incidence of pouchitis. This is based on prior data that abdominal fat secretes cytokines and adipokines involved in the intestinal immune response. Gao *et al*. studied this in a sample of 277 UC patients and 40 FAP patients who had undergone pouch formation. CT imaging taken prior to pouch failure was used to compare the two groups in terms of peripouch fat. It was found that patients with UC had higher total peripouch fat ($p = 0.030$) as well as a higher incidence of pouchitis (58.5% *versus* 15.0%, $p < 0.001$).[9] Despite the observational nature of the data as well as the unbalanced sample size, this study suggests an association between pre-existing peri-pouch fat and the risk of developing pouchitis, which may be related to the proinflammatory effects of the former.

Furthermore, the extent or severity of colitis may serve to predict the risk of subsequently developing pouchitis after surgery. This has been assessed prospectively, where patients who had acute severe UC, as well as pancolitis, were more likely to develop both acute and chronic pouchitis on follow-up.[11,12] As alluded to above, these findings suggest that a pre-existing severe inflammatory state can increase the risk of developing pouchitis. This may serve as a predictive tool to determine which patients may be at higher risk post-surgery.

Going a step further, histology from the colectomy specimen at the time of surgery has the potential to inform the risk profile of the patient with regard to pouchitis. Multiple studies have undertaken a retrospective assessment of colectomy specimens from patients who underwent pouch surgery, with mixed results. For example, Araki *et al.*[13] found that histological features such as mononuclear cells and eosinophil infiltration had a utility in predicting the development of chronic pouchitis. On the contrary, a similar study by Nasseri *et al.*[14] found no single atypical histopathological feature of UC, or combination of features, was associated with any adverse pouch outcome. Note should be made regarding the vast differences in histological and anatomical criteria used between these studies, which may account for the differences in outcomes. Although the current research is equivocal, a better understanding of the pathophysiology of pouchitis may help to delineate which histological features are most sensitive in predicting the future risk of pouchitis.

The presence of extraintestinal manifestations (EIMs) of UC may be an additional risk factor for the development of pouchitis in this patient population. These include EIMs that correlate with the level of UC activity (such as uveitis) as well as those not clearly related to disease activity such as primary sclerosing cholangitis.[15] While their pathophysiology is poorly understood, it is thought to be an autoimmune response at extraintestinal sites due to shared epitopes with the affected colonic mucosa. A 2019 meta-analysis by Hata *et al.*[15] assessed the correlation between EIMs and pouchitis. Assessing data from 22 observational studies, it was found that the presence of any EIMs in UC was significantly associated with the development of both acute and chronic pouchitis. It is unclear from this data if more severe EIMs, such as Primary Sclerosing Cholangitis (PSC), are more strongly correlated with pouchitis than comparatively less severe ones, such as arthralgias. What it does suggest, however, is that pouchitis may be a manifestation of the underlying immune dysregulation present in UC.

Surgical factors

Surgical factors that influence the risk of pouchitis include the type of anastomosis (hand-sewn), the proximity of the anastomosis to the

dentate line (<0.5 cm) and the method of pouch construction.[16,17] Additionally, a two-stage operation for the formation of IPAA is associated with a higher risk of pouchitis than a three-stage surgery ($p < 0.05$).[16] However, these findings remain observational at this stage and an encompassing pathological reason for the increased incidence of pouchitis in these patients remains to be found.

The use of anti-inflammatory medication Non-steroidal anti-inflammatory medications (NSAIDs), the presence of iron deficiency anaemia, thrombocytosis and a longer duration of follow-up after pouch construction are associated with an increased risk of the development of pouchitis.[17–20] The preoperative use of steroids has been shown to increase the risk of developing acute pouchitis almost four-fold but was not associated with the development of chronic pouchitis.[17]

Patient modifiable risk factors for pouchitis

Smoking

Although smoking is a predisposing factor for Crohn's disease, it is protective for the development of UC.[21,22] The association between pouchitis and smoking history appears to be conflicting. In a prospective multivariate analysis of clinical factors associated with pouchitis, Fleshner *et al.*[23] found that smokers had an almost two-fold increased risk of developing acute pouchitis but a reduced risk for chronic pouchitis (antibiotic dependent and antibiotic refractory). Similar studies have demonstrated a higher risk for pouchitis associated with smoking; however, the same theory has not been confirmed in other studies.[24–27]

Dietary factors

Research on the impact of diet on the development of pouchitis has gained momentum over the last decade as dietary intake has been shown to change the composition of the gut microbiome.[28] Ianco *et al.*[29] investigated the effect of dietary intake of 80 patients compared to healthy controls. Amongst the pouch patients, those who had no evidence of pouchitis were found to consume twice as many servings of fruits (3.6 ± 4.1 *versus* 1.8 ± 1.7 servings/day, respectively, $p < 0.05$). In the same study, patients who had pouchitis were found to have less intake of lipid-soluble antioxidants, vitamin A and vitamin C19. The authors of this study suggested that decreased consumption of antioxidants resulted in increased oxidative stress, thereby resulting in the manifestations of pouchitis.

Croagh *et al.* evaluated the effect of low FODMAP (fermentable oligo-, di- and monosaccharides and polyols) diets on bowel function in pouch patients. These molecules are poorly absorbed in the small intestine, and thus produce an increased osmotic load on the pouch, resulting in increased frequency of loose stools. The small retrospective study evaluated the effect of a low FODMAP diet on seven patients with ileal pouch or ileo-rectal anastomosis, two of whom had been previously diagnosed with chronic pouchitis. Whilst the five non-pouchitis patients had significant improvement in self-reported stool frequency and consistency, the two patients with chronic pouchitis did not show a response to dietary change.[30] The authors posit that the increased irritability and secretory state associated with pouchitis may preclude the potential benefits of a low FODMAP diet.

Inulin is a long-chain fructan thought to reduce gut inflammation by enhancing the growth of indigenous gut flora. Zella *et al.* studied the effect of inulin on reducing subclinical inflammation in a group of UC and FAP patients with an IPAA.[31] In this double-blinded crossover design study, patients with an IPAA, without clinical pouchitis, were randomized to an inulin supplemental diet (24 g/day) or placebo over a 3-week period. Both groups of patients underwent endoscopy as well as faecal analysis at the end of the 3-week period. Histologic and endoscopic inflammation was judged using the corresponding criteria from the Pouchitis Disease Activity Index (PDAI) scoring system. Compared to placebo, the patients randomized to supplementary inulin were found to have an increased luminal content of short-chain fatty acids (SCFA) butyrate (fermentation product of inulin) with a lower PDAI histological and endoscopic scores and reduction in *Bacteroides fragilis*. Thus, inulin supplementation results in a reduction in pouch inflammation and may reduce the risk of developing pouchitis.[32]

Gut microbiome as a risk factor for pouchitis

The gut microbiome, consisting of a vast array of microorganisms, plays an important role in both metabolic and immune processes within the small and large bowel. Variations in its make-up and

function have thus been implicated as risk factors for pouchitis development. These risk factors will be touched on here and then elaborated further later in the review.

Microbiome as a predictor of pouchitis

The mucosal and faecal microbiota of UC patients with pouchitis compared to patients with FAP and healthy UC pouches is distinctly different.[31,33–35] The presence of specific bacterial species (i.e. *Ruminococcus gnavus*, *Bacteroides vulgatus* and *Clostridium perfringens*, and the absence of *Blautia* and *Roseburia*) in faecal samples was reported to predict a higher risk of pouchitis in UC patients prior to IPAA formation.[36] Higher bacterial diversity was also found to be a predictive factor and was detected up to 1 year prior to inflammation.[36] In a 2021 study, Dubinsky *et al.* aimed to develop a model that can distinguish between patients with a normal pouch and those with pouchitis based on faecal samples. While no single bacterial species or function was found to be highly discriminative, models based on top-ranking species (71.4% accuracy) and top-ranking bacterial enzymes (71.4% accuracy) performed better than a metabolic pathway-based model.[37]

Bile acids

The role of bile acids in the development of pouchitis has gained considerable interest over the past decade. To simplify, lithocholic acid and deoxycholic acid are normally the most abundant gut secondary bile acids (SBAs) and are thought to be an important modulator of intestinal inflammation. They are derived from primary bile acids in a process dependent on intestinal microbes found primarily in the colon (see Figure 2). Thus, any procedure that disrupts the colonic microbiome (such as IPAA surgery) would result in a decreased level of SBAs. Moreover, Hakala *et al.*[38] found that patients with an IPAA also had impaired cholesterol absorption, which can further explain the deficiency in SBA. It follows then that this intestinal inflammation can potentially be ameliorated by SBA restoration.[39]

Historical context regarding aetiology

Bacterial theories

Some of the earliest longitudinal studies found that the ileal pouch flora shifts significantly over time, with colon-predominant and anaerobic species increasing and ileum-predominant species decreasing after stoma closure.[34,40] While no specific species has been identified as a sole culprit, overall decreased bacterial diversity has been demonstrated in cases of pouchitis compared to healthy pouches.[41–43]

Several different theories exist pertaining to the microbiological aetiology underlying pouchitis. Studies have pointed to changes in the microbiome being associated with pouchitis, specifically a reduction in the ratio of anaerobic to aerobic bacterial counts.[44] Some studies have identified elevated counts of *C. perfringens* and reduced counts of *Lactobacilli* and *Bifidobacteria* in pouchitis.[45] *C. perfringens* is a mucin-degrading bacteria, whereas *Lactobacilli* and *Bifidobacteria* are known to inhibit the growth of potentially pathogenic bacteria, which may explain the role changes in their respective population numbers plays in the aetiology of pouchitis.[46]

Furthermore, sulphate-reducing bacteria (SRB), such as Desuflovibrio and Bilophila, have been found to occur more abundantly in UC pouches compared to FAP.[46] It has therefore been suggested that SRBs may be implicated in the aetiology of pouchitis, given it occurs most commonly in pouches of patients with a background of UC.[47] It should be noted however that this correlation is not linear, with SRBs existing in approximately 80% of pouches, while the incidence of pouchitis is lower.[44] Other patterns of bacterial colonization associated with pouchitis include a greater anaerobe-to-aerobe ratio and increased facultative anaerobe counts.[47] In another study, species significantly enriched in pouchitis compared to normal pouch samples included *Escherichia coli*, an unclassified *Providencia* species, *Yersinia* species and an unclassified *Acinetobacter* species.[48]

The early observations that there are bacterial shifts in the pouch may have provided the rationale to explore antibiotics as a treatment for pouchitis. Since these observations, antibiotics have demonstrated both clinical improvements in the treatment of pouchitis as well as changes in the microbiome. Combination antibiotic therapy for patients with chronic, treatment-resistant pouchitis resulted in clinical improvement with an associated significant decrease in total anaerobes, aerobes, *Enterococci*, *Lactobacilli*, *Bifidobacteria* and *bacteroides.*[49] Gosselink *et al.* examined the

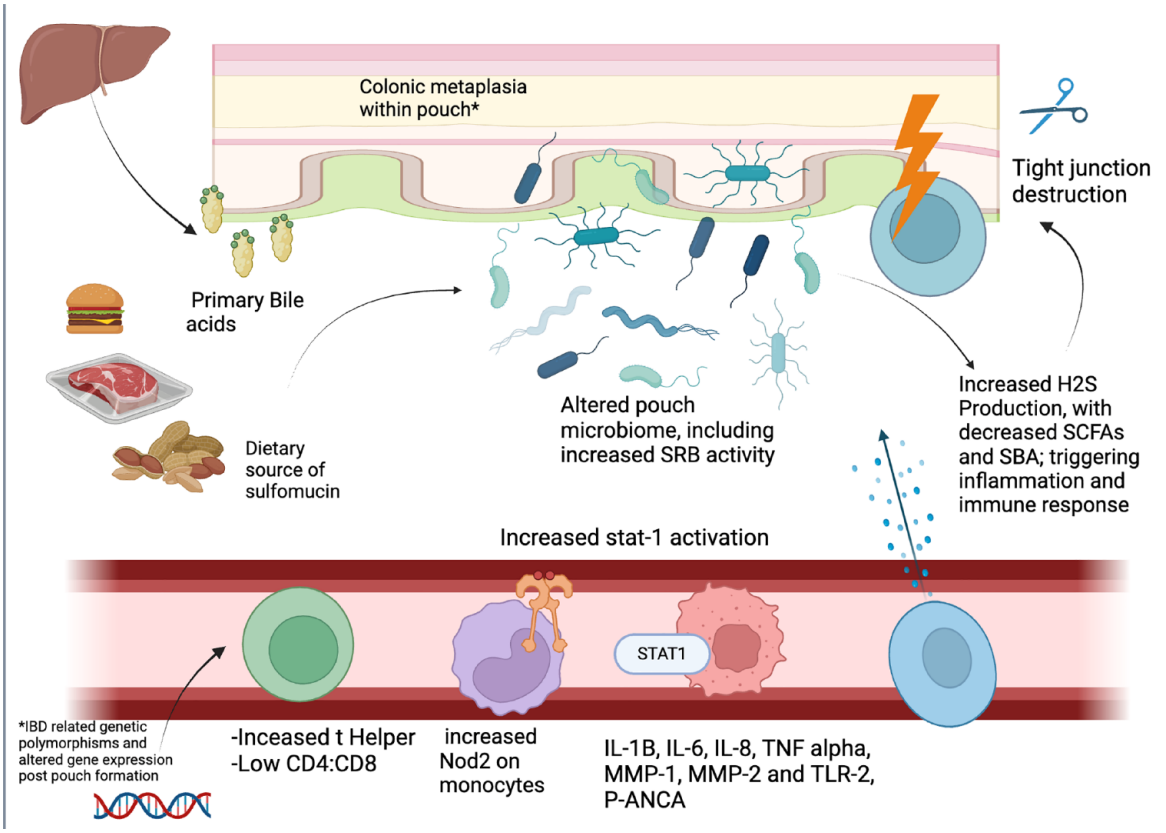

Figure 2. Summary of genetic, immune and microbiome factors involved in the aetiology of pouchitis. The altered pouch microbiome leads to increased activity of sulphate-reducing bacteria; which convert sulphomucin to hydrogen sulphide within the pouch. This, along with reduced levels of anti-inflammatory substances such as short-chain fatty acids and secondary bile acids, causes oxidative damage as well as increased epithelial permeability, leading to an increased immune response with subsequent inflammatory change. Processed foods rich in sulphite preservatives may contribute to this process by increasing the amount of sulphomucin substrate. Also illustrated is the effect of inflammatory bowel disease-related genetic polymorphisms as well as altered gene expression within the colonic mucosa, which may result in a proinflammatory state that then contributes to pouchitis development.

faecal flora of 13 UC patients with pouchitis, before and during treatment with either ciprofloxacin or metronidazole, as well as during pouchitis-free periods. These participants were noted to have faecal flora resembling normal colonic flora during pouchitis-free periods, with increased anaerobes and no or low numbers of pathogens. During pouchitis episodes, anaerobe counts decreased and aerobic bacterial counts increased, with increased numbers of pathogens such as *C. perfringens* and haemolytic strains of *E. coli*. Metronidazole treatment eradicated *C. perfringens* and reduced the overall number of anaerobes. Ciprofloxacin was found to eradicate both *C. perfringens* and haemolytic strains of *E. coli*, while overall anaerobe counts were maintained.[50]

Since pouchitis may respond to antibiotics, toxic bacterial products have been proposed as likely candidates in the aetiology of this condition. In a 2009 review, Coffey *et al.* provide a multi-factor theory of the pathogenesis of pouchitis. Increased production of sulphomucin, a metabolic substrate for SRB, occurs due to colonic metaplasia in the pouch. This then leads to colonization by SRBs, which produce hydrogen sulphide (H_2S).[51] This molecule has been observed to be increased in cases of active pouchitis and reduced with antibiotic therapy.[52] H_2S may have toxic effects in the gut by increasing epithelial permeability, reducing barrier function and contributing to cellular oxidative stress causing DNA damage (see Figure 2).[53,54] Sulphomucin expression is increased in UC pouches compared to FAP,

raising the possibility that baseline inflammation may be an important driver for colonic metaplasia and the subsequent downstream effects described above.[55] As well, this has led to theories that reducing dietary intake of foods rich in sulphate preservatives can reduce levels of sulphomucin, which would then reduce the risk of developing pouchitis.[46]

More recently, specific metabolic by-products of the pouch bacteria have been explored as contributors to the pathogenesis of pouchitis. Specifically, SCFA, which is a fermentation by-product of anaerobic bacteria, has been theorized to play an important role in colonocyte metabolism, barrier function and the suppression of pathogenic bacterial species.[37,47] Patients with pouchitis have previously been shown to have reduced faecal levels of SCFA compared to patients with a normal pouch.[56,57] Dubinsky *et al.* examined the metagenomes derived from faecal samples of patients with pouches. They found that pathways and species that produce butyrate, a SCFA known to have anti-inflammatory properties and the preferred energy source for colonocytes, were decreased in pouchitis.[37]

Interplay between genetics, immunology and microbiome on the impact on pouchitis

The fact that pouchitis seems to occur almost exclusively in patients with underlying inflammatory bowel disease (IBD) suggests a contribution of genetics as a risk factor. More specifically, studies have demonstrated the presence of genetic polymorphisms in immunoregulatory cytokines and antigen receptors in patients with pouchitis.[58,59] As with IBD, this may be related to a loss of tolerance to bacterial as well as autoantigens, which then raises the risk for the development of pouchitis. This will be explored in further detail in this section.

Interleukins

Interleukin (IL)-1B pro-inflammatory cytokine is produced by monocytes and is thought to have a role in autoimmunity with studies demonstrating that IL-1B expression is increased in active UC.[58,60] A single-centre Japanese study analysing IL-1B association with the development of pouchitis showed that the T allele (rs1143627) was associated with the development of pouchitis.[61] This finding remained significant even after the study adjusted for confounding factors. However, it must be noted that like many other similar studies analysing genetic association with pouchitis, it was limited by ethnic homogeneity. Interestingly, the IL-1B gene is next to the interleukin receptor antagonist (ILRA1) gene and the two demonstrate a strong linkage disequilibrium, suggesting that an interplay between these two genes may be implicated in the development of pouchitis.[62]

The IL-1 receptor antagonist (IL1RA) competitively binds to the IL-1 receptor, impeding the binding of IL-1 and subsequently preventing downstream pro-inflammatory signalling. There has been some evidence suggesting that an intestinal mucosal imbalance of these two factors, IL-1 and IL1RA, may contribute to the chronic inflammatory state in UC (see Figure 2).[63,64] The associated variable number tandem repeat allele 2 of IL-RA (ILRA*2) is associated with UC.[62] While Aisenberg *et al.*[65] demonstrated an inverse relationship between ILR*2 and pouchitis in UC patients. Two other studies have demonstrated a positive association. The first by Brett *et al.*[66] demonstrated that among UC IPAA patients, there were higher carriage rates of ILRA*2 compared to patients with FAP IPAA patients, particularly among UC IPAA patients with pouchitis. A subsequent study by Carter *et al.*[62] assessed UC patients undergoing colectomy and demonstrated that 57% of these patients carried at least one copy of the IL-RN*2. Like the Brett *et al.* study, this study also showed that patients who developed pouchitis had higher rates of carriage of ILRA*2 compared to those without pouchitis, an association that remained significant even after adjusting for confounding factors. These studies provide supporting evidence for the ILR*2 mutations as a genetic risk factor for the development of pouchitis.

Toll-like receptors

Toll-like receptors (TLR) are a group of 10 pattern recognition receptors that are expressed on a variety of effector cells, including macrophages and dendritic cells. They are vital to the innate immune system's recognition of microbial antigens, including lipopolysaccharides of gram-negative bacteria. Given their role in the innate immune system and the hypothesis that immune imbalance may lead to pouchitis, investigations into TLR expression patterns and mutations have been undertaken. TLR is expressed in normal

ileal mucosa (see Figure 2)[67] and, additionally, it has been demonstrated that compared to normal ileum, there is differential expression of TLR within pouch tissue.[68–70]

Yuji et. al. analysed the expression of TLR 2, 3, 4 and 5 in a small number of healthy ileal biopsies from colon cancer patients and compared them to patients with UC undergoing IPAA. They demonstrated that patients with normal ileal tissue (colon cancer group) expressed TLR3 and TLR5. However, TLR3 and TLR5 were not detectable within pouch mucosa from UC IPAA patients. Additionally, while patients with a healthy pouch demonstrated expression of TLR4, among patients with pouchitis TLR2 and 4 were very strongly up-regulated.[69] Of note, previous studies have shown that TRL4 is up-regulated in the intestinal epithelial cells in UC patients.[68] In a later study by Heuschen *et al.*, of UC patients undergoing IPAA, TLR from ileal biopsy upstream of the pouch was compared to pouch biopsies of the same patient on routine follow-up. This study demonstrated that within active pouchitis mucosa, there was a decrease in TLR3 expression and an increase in TLR5 expression, compared to upstream ileal biopsies and healthy pouch mucosa.[70]

Subsequent studies have gone on to analyse the genetic polymorphism within TLR among patients with pouchitis. An Italian study, by Lammers *et al.*, analysed various single nucleotide polymorphisms (SNPs) within TLR4, TLR9, CD14 among UC patients with IPAA compared to health controls. They demonstrated an increased frequency of TLR9-1237C allele in patients with chronic relapsing pouchitis compared to those with infrequent pouchitis.[71] Ferrante *et al.*[67] evaluated 50 SNPs within TLR1–10, among 144 UC patients post-IPAA (80 of whom had pouchitis), and found that one independent risk factor for the development of pouchitis was the GT/TT genotype at TLR1 S871. These studies highlight the importance of the innate immune system in the pathophysiology of pouchitis and contribute evidence that immune dysregulation may be a causative factor in its development.

NOD2/CARD15

The NOD2 gene (also known as CARD15) transcribes the NOD2 protein, an intracellular pattern recognition protein, that is integral to the innate immune system. It is involved in activating the pro-inflammatory NF-κB pathway upon recognition of muramyl dipeptide (MDP) found on the cell wall of some bacteria (see Figure 2). One of the most significant genetic risk factors for Crohn's disease identified to date has been several SNPs within the NOD2/CARD15.[72,73]

Given this association, several studies have been conducted analysing various NOD2 gene polymorphisms associated with pouchitis, yielding heterogeneous results. Roughly half of these studies have failed to find any association with a variety of NOD2 polymorphism and pouchitis.[67,71,74] However, it may be argued that these negative studies were underpowered to demonstrate a significant association.

Three studies to date have found a positive association between the development of pouchitis and CARD15 mutations.[75–77] The largest of these was a multicentre study that demonstrated that NOD2 polymorphism, particularly the NOD2insC (rs2066847, also known as CARD15 L1007fsinsC) was associated with severe or frequent episodes of pouchitis.[77] This polymorphism, which is the most consistently associated with Crohn's disease, impairs the ability of the NOD2 protein to bind bacterial MDP.[78,79] A similar study by Meier *et al.* also demonstrated that the NOD2insC polymorphism was found in a higher frequency of patients with severe pouchitis compared to those without. However, this study was unable to show a difference in NOD2 polymorphism between patients with mild pouchitis compared with healthy controls, both of whom had a frequency of NOD2 mutations.[76]

Schieffer *et al.* reported one small case study of a single family with FAP, with two siblings who both had ileoanal anastomosis (IPAA) at a similar age, one of whom subsequently developed pouchitis. Pouchitis among FAP patients undergoing IPAA is rare. Interestingly, the sibling who went on to develop pouchitis was a carrier for NOD2 polymorphism (rs17221417 and rs2076756).[80] This provides further support for the role of NOD2 as a predisposing factor for the development of pouchitis.

Serological markers

The two major serological markers representing a spectrum of IBD include anti-*Saccharomyces*

cerevisiae antibodies (ASCA) and antineutrophil cytoplasmic antibodies (ANCA).[81] Mitsuyama *et al.*[82] completed a meta-analysis on the relationship between ANCA status and pouchitis, patients who were ANCA positive had an OR of 1.76 of developing chronic pouchitis (but not acute pouchitis). In the same study, ASCA positivity was not associated with acute or chronic pouchitis. Other serological markers associated with pouchitis include high serum IgG4, autoantibodies to bacterial antigens as well as the host's self-tissue (anti-CBir1 flagellin antibody).[11,83–87]

Perinuclear antineutrophil cytoplasmic antibodies

Perinuclear antineutrophil cytoplasmic antibodies (p-ANCA) is positive in approximately 40–80% of patients with UC and tends to be associated with a more aggressive phenotype.[88–90] While there have been a considerable number of studies into the relationship between p-ANCA and pouchitis, the results of these studies are contradictory. These contradictions may be due to the incongruous definition of pouchitis used in each study, with differing clinical definitions of disease state and variable use of histology, making the overall message relating to p-ANCA difficult to interpret.

Several of the studies failed to demonstrate an association between p-ANCA and pouchitis. Aisenberg *et al.*[91] compared patients with refractory pouchitis (including IPAA patients and patients with Kock pouch ileostomy) to matched controls without pouchitis, with no difference found. While Yasuda *et al.*[92] showed that patients with UC demonstrated higher p-ANCA levels than non-UC patients, they were unable to show any significant difference in p-ANCA levels between UC IPAA patients with or without pouchitis. Additionally, several other papers failed to demonstrate any association between these groups, including among a paediatric population.[65,66,93,94]

Conversely, other studies have demonstrated a positive correlation between pouchitis and p-ANCA. Yang et. al. analysed post-operative p-ANCA in a group of UC IPAA, demonstrating higher p-ANCA levels among patients who had more frequent relapses of pouchitis. This study also demonstrated higher p-ANCA levels among active or recent attacks when compared to those for whom it had been a year or more since suffering an episode of pouchitis.[95] Sandborn *et al.* analysed the extremes of the spectrum by comparing chronic pouchitis patients to UC IPAA without pouchitis, and a healthy patient control group. In doing so, they were able to demonstrate a positive correlation between p-ANCA and chronic pouchitis.[96] Vecchi *et al.*[97] also found a similar result in a smaller study, showing a higher frequency of p-ANCA among all pouchitis patients compared to patients with a healthy pouch.

All of these studies have been retrospective with p-ANCA levels taken post-operatively. However, more recently, a study by Fleshner *et al.* performed a prospective study using preoperative p-ANCA levels. This study concluded that, while pre-operative p-ANCA expression alone does not predict the subsequent occurrence of pouchitis, higher levels of preoperative p-ANCA (100 EU/mL) appeared to be predictive of the development of chronic pouchitis. The relative risk of developing chronic pouchitis among patients with a higher p-ANCA level was more than eightfold (hazard ratio 8.47; 95% confidence interval 1.67–16/95) compared to those with low or medium levels of p-ANCA.[87] So, while there may be conflicting evidence regarding the association of pouchitis with p-ANCA, this study highlights the potential use of the p-ANCA level as a pre-operative tool to risk-stratify patients who may develop pouchitis. Further evidence may be needed to validate this finding.

Gene expression post pouch formation

Whereas the presence of gene and gene mutations, such as NOD2, is a risk factor for pouchitis, it has also been demonstrated that how genes are expressed may have a role in the development of pouchitis. Variations in gene expression may play a role in the heterogeneous presentations of pouchitis, resulting in several different phenotypes. A 2022 study by Akiyama *et al.*[98] proposed the 'Chicago classification' for pouchitis based on endoscopic findings and anatomical location of disease; resulting in seven distinct phenotypes: (1) normal, (2) afferent limb involvement, (3) inlet involvement, (4) diffuse, (5) focal inflammation of the pouch body, (6) cuffitis and (7) pouch with fistulas noted 6 months after ileostomy takedown. Of these, the diffuse phenotype was associated with the poorest long-term outcomes.

There is evidence that the transcriptome of the pouch mucosa compared to the mucosa of the pre-pouch ileal sample is significantly different.[99–101] With studies demonstrating that pouch mucosa evolves physiologically and functionally to resemble colonic mucosa post-operatively.[102]

The interplay between changes in the whole transcriptome, pouchitis and the microbiome has been studied by Morgan *et al.*, who demonstrated that while the transcriptome differed significantly between pre-pouch ileal samples and pouch samples, there was no significant variation reflected in the microbiome. The most substantial impact on the microbiome appeared to stem from antibiotic use, while the presence of pouchitis or the tissue location (pre-pouch *versus* pouch) had minimal effect.[99]

Ben-Shachar *et al.* demonstrated that changes in the gene expression within pouch mucosa among patients with UC were more substantial than those of FAP patients undergoing IPAA. Additionally, it was demonstrated that the magnitude of changes in gene expression was largest amongst patients with Crohn's-like disease of the pouch, with smaller changes demonstrated among chronic pouchitis patients and even less in the healthy pouch mucosa of UC patients.[103] However, it was noted that there was a significant overlap of the observed gene expression changes between these three different groups, indicating a spectrum of disease rather than distinct disease states. Studies of specific genes, rather than the whole transcriptome, have demonstrated an increase in particular gene expression, including IL-1B, IL-6, IL-8, Tumor necrosis factor (TNF) alpha, Mucous membrane pemphigoid (MMP-1), MMP-2 and TLR2 in active pouchitis compared to normal pouch mucosa.[58,70,104]

While these studies provide some insight into the pathogenesis of pouchitis, the observed differences in gene expression are limited, as they do not indicate whether the changes observed are causative of pouchitis or a consequence of the disease state. Further genomic analysis based on classifications, such as the aforementioned Chicago system, may provide a clue as to the relationship between host/microbiome transcriptome and expressed pouchitis phenotype.

Conclusion

The aetiology of pouchitis remains poorly understood but evidence supports the interaction between genetics, the immune system and the environment as possible drivers of pouchitis. Integrating some of the newer technologies may help us understand how all these individual risk factors impact on the pouch which ultimately may help guide treatment and management of pouchitis.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable. Both Figures 1 and 2 are designed and illustrated by JPS, who approves of them for use in this publication.

Author contributions

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