

Review Article

Pouchitis: insight into the pathogenesis and clinical aspects

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Abstract: Ulcerative colitis (UC) is a chronic intestinal inflammatory disease and familial adenomatous polyposis (FAP) is an autosomal dominant inherited disease. Both diseases, despite being different, may require the same surgical procedure: proctocolectomy with ileal pouch-anal anastomosis (IPAA). The main complication after this procedure is pouch inflammation (pouchitis). This inflammatory complication can affect up to 60 percent of patients who receive IPAA for UC, and a very small percentage of the FAP patients. The purpose of this review was to determine the current molecular mechanisms in its pathogenesis and detail the risk factors involved in pouchitis, its diagnosis, and treatment.

Keywords: Ulcerative colitis, familial adenomatous polyposis, ileal pouch-anal anastomosis, pouchitis

Introduction and general aspects of ulcerative colitis and familial adenomatous polyposis

Ulcerative colitis (UC) represents a chronic intestinal inflammatory disease with a non-fully understood etiology. The disease is characterized by periods of relapse and remission, and it usually affects the rectum, and a continuous extension of the colon, often with an abrupt transition between the inflamed and non-inflamed mucosa. Given that the use of biological drugs has provided more efficient management for patients, the rates of UC-related surgeries in the last decades have decreased remarkably. However, UC patients are prone to complications such as toxic colitis, intestinal bleeding, dysplasia, and cancer [1].

Familial adenomatous polyposis (FAP) is an autosomal dominant inherited disease, characterized by the formation of hundreds to thousands of adenomatous polyps in the large intestine and rectum. Generally, FAP arises early in life, which can lead to colon cancer in most patients before the age of 40. The etiology of FAP is based on a genetic mutation in the

tumor suppressor gene, called Adenomatous Polyposis Coli (APC), which predisposes to the development of colorectal cancer [2, 3].

Despite being different, both diseases may require the same surgical procedure. Proctocolectomy with ileal pouch-anal anastomosis (IPAA) is the elective procedure of choice in the surgical management of refractory UC and FAP with many polyps in the rectum. Twenty-five to thirty percent of UC patients undergo IPAA. The objectives of this surgical approach focus on avoiding malignant degeneration and promoting definitive treatment, as well as maintaining fecal continence [4]. The main IPAA-related complication is pouch inflammation (pouchitis) which can affect up to 60% of UC and a very small percentage of FAP patients [4-6].

IPAA has positively altered surgical management of both diseases, making it possible to avoid a permanent stoma [4-6]. In this review we will address the molecular aspects of pouchitis, showing the differences between UC and FAP. We also will highlight the risk factors involved and the general aspects of treatment.

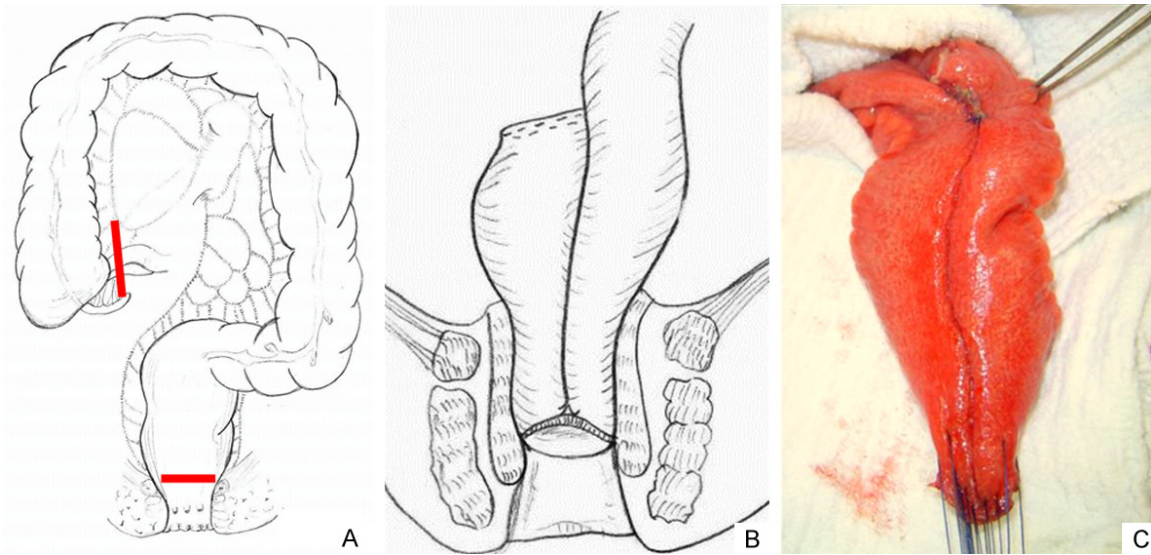


Figure 1. Proctocolectomy and ileal pouch-anal anastomosis (IPAA). A. Illustration showing the extension of the intestinal resection. All large intestine and rectum are removed, as evidenced in the follow-up between the red lines. B. Restorative proctocolectomy with J-shaped pouch and ileal pouch-anal anastomosis. C. Surgical aspect of an ileal J-shaped reservoir during a procedure, before performing a handsewn anastomosis (Colorectal Surgery Unit, Unicamp).

Surgical technique, clinical aspects, and functionality of the ileoanal pouch procedure

The first report of the ileal reservoir dates back to 1933. Since then, several complex surgical procedures have been attempted without much success. The great evolution in pouch surgery occurred in 1978 when Sir Alan Parks and Mr. John Nicholls from St. Mark's Hospital in London agreed to carry out a three-limbed ileal reservoir with ileoanal anastomosis, creating the first IPAA. They fashioned an S-shaped pouch for a patient with UC after proctocolectomy [4, 6].

After that, the surgical approach to pouch formation advanced rapidly and several types of reservoirs were developed, such as "J", "W", and "S" pouches. As described in 1980 by Utsunomiya et al., the J-pouch is the most common type of ileal pouch, and this preference is mainly due to its superior storage and emptying function over the S or W-pouch configurations [5, 7].

The surgery may require one, two, or three stages, including the creation of a temporary diverting ileostomy in order to minimize the complications of an ileal pouch fistula [6]. Over the years, there has also been a progression in the way

the anastomosis is made. Nowadays, surgeons prefer a stapled anastomosis rather than a hand-sewn one. While handsewn anastomosis permits mucosectomy (the original description of the technique included a mucosectomy followed by a handsewn anastomosis between pouch and anus), that removes a potentially inflammatory rectal mucosa, at the same time it increases the risk of the anal sphincter and anal transition zone (AZT) damage, which is an important sensory-rich area of the anal canal that allows flatus/stool discrimination. A stapled IPAA, on the other hand, avoids a mucosectomy. In addition, it is quicker to perform and can offer a better functional outcome with lower nocturnal seepage and incontinence. As the advancement of technologies progresses, laparoscopic, robotic, single incision laparoscopic surgery (SILS), and transanal proctectomy (TaTME) have also been used for performing IPAA. These new techniques promise to improve surgical accuracy, postoperative recovery, and postoperative pain. However, more studies are needed [4, 6, 8]. **Figure 1** illustrates the IPAA procedure.

A main advantage of IPAA is the avoidance of a permanent ileostomy. Most UC patients who undergo IPAA report good quality of life after surgery with an average of six bowel move-

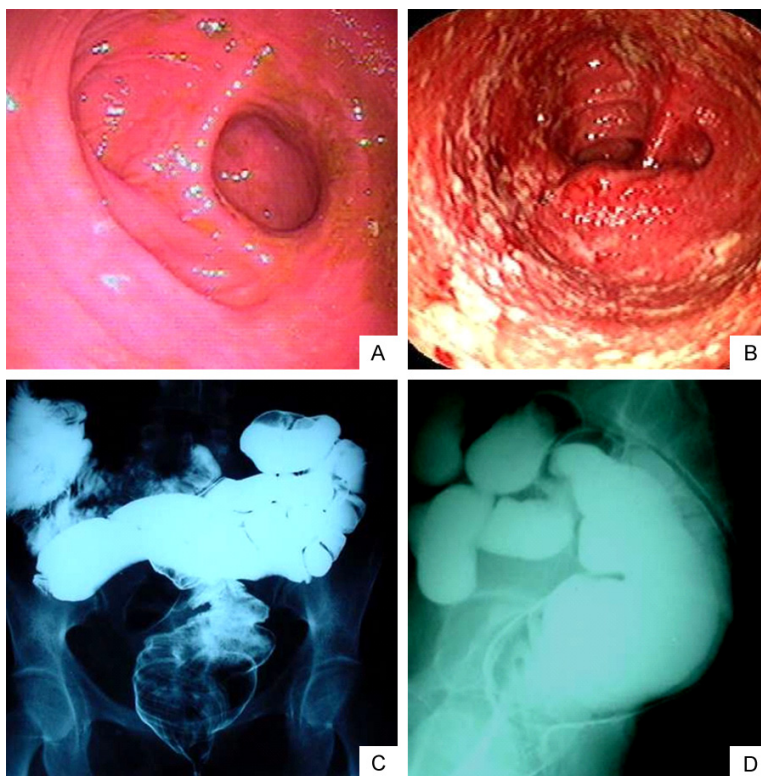


Figure 2. Endoscopic and radiological aspects of the ileal pouch. Endoscopic image of patients showing in (A) Normal mucosal appearance and in (B) Abnormal mucosa suggesting pouchitis. The endoscopic elements of the ileal pouchitis are granularity, loss of vascular pattern, edema, friability, mucosal hemorrhage, and superficial ulcers (Endoscopy Unit, Gastrocenter, Unicamp). Radiological aspects of the ileal pouch in the pouchogram, in (C) Frontal view (posteroanterior) and (D) Lateral view evidencing no abnormalities performed before ileostomy closure (Radiology Unit, Unicamp).

ments per day, due to the improvement of the underlying disease. Overall, ileal pouch function is reported to be stable over time [9-11].

The evaluation of these patients must be strict and numerous tests contribute to the assessment and monitoring of expected complications. Pouch endoscopy is useful for assessing inflammatory changes in the pouch (granularity, loss of vascular pattern, edema, friability, mucosal hemorrhage, contact bleeding, and superficial ulcers) and it also allows the assessment of pre-pouch ileum and rectal cuff, if present. Furthermore, this technique also allows biopsies to be performed when necessary [10, 11].

In addition to pouch endoscopy, pouchgram is another evaluating exam, which is useful to evaluate strictures, long efferent limb, decreased pouch complacency, and emptying.

The use of a pouchogram before ileostomy closure should be indicated for cases of suspected clinical complications. However, pouchogram's sensitivity to predict complications following ileostomy closure in patients after IPAA is rather low and it rarely changes the management of these patients in clinical practice [6, 12]. **Figure 2** shows the endoscopic and radiological characteristics of the ileal pouch.

Although the mortality rate for IPAA is low, several pouch-specific complications following this surgery are common, such as hemorrhage, acute pelvic sepsis, anastomotic leak, infected hematoma, portal vein thrombus; as well as late complications, including chronic pelvic sepsis, pouch fistulae, small bowel obstruction, pouch dysfunction, cuffitis, dysplasia/malignancy, infertility and pouchitis [4, 6, 8].

Theoretically, once the colon and rectum are removed, either FAP or UC patients experience an improvement in the symptoms of the disease. However, about 50% of UC patients who have undergone IPAA develop at least one episode of subsequent pouchitis [6, 8]. Pouchitis is an idiopathic nonspecific inflammation confined to the ileal pouch that leads to symptoms similar to that of UC, including increased frequency of bowel movements, abdominal cramps, straining during defecation, incontinence, hematochezia, mucous and/or bloody exudates, fever, abdominal pain and urgency [4, 6, 8].

In a series of publications and clinical follow-ups of PAF and UC patients, it was observed that both diseases may develop pouchitis after IPAA. Although the first episode of pouchitis can occur following ileostomy closure, the patients can develop it within the first year after the ileoanal reservoir procedure [4, 6, 8]. The definitive diagnosis is made in combination

with clinical, endoscopic, and histopathological findings. The endoscopic appearance of pouchitis resembles the colorectal inflammation of UC. The main score used in the literature is the Pouchitis Disease Activity Index (PDAI), in which demographic data and scored symptoms are collected and graded [13]. Biopsies taken from the posterior wall of the pouch (if the pouch has a normal endoscopic appearance) or from inflamed areas are accessed together with scored symptoms. A total PDAI of seven or more indicates the presence of pouchitis. Symptomatic patients with no endoscopic and histologic evidence of pouchitis, besides a PDAI less than seven points, indicate the absence of pouchitis [9, 13-15].

Although pouchitis can also be seen in FAP patients who undergo the same IPAA procedure, they rarely develop this inflammatory condition of the pouch. The etiology of pouchitis remains unclear. However, the difference in the percentage of involvement between UC and FAP points to molecular and immunological mechanisms, which may underlie this inflammation in ileal reservoir, mainly in UC.

Pouchitis: insight into the molecular mechanisms involved in the pathogenesis

Several factors have been suggested to influence the genesis of this complication, such as immune alteration, genetic susceptibility, autophagy-associated epigenetic changes, environmental factors, fecal stasis, bacterial overgrowth, dysbiosis, deprivation of short-chain fatty acids (SCFA), mucosal ischemia-reperfusion, and oxygen radicals in ischemia-induced lesions [8, 10, 11]. Many of these factors were evidenced not only in UC but also in FAP patients with IPAA, hampering their interpretation as isolated causes of primary pouchitis. In this section, we will discuss such factors. **Table 1** illustrates the most relevant studies that deal with the potential factors involved in the primary pouchitis etiology.

Immune system dysfunction

Toll-like receptors (TLRs) comprise a class of proteins that play a key role in the innate immune system and intestinal epithelium defense. These receptors are responsible for recognizing bacterial lipopolysaccharides from both commensal and pathogenic bacteria.

Several studies have shown a variation in gene expression of these receptors in the context of inflammatory bowel diseases (IBD) [16-24]. Toiyama *et al.* (2006) demonstrated that TLR2 expression was upregulated in patients with pouchitis, whereas TLR4 expression was increased in both normal pouches and pouchitis [17]. Similarly, using *in vitro* experiments in which macrophages were stimulated with TLR bacteria and ligands, Rahman *et al.* (2010) demonstrated that the response associated with TLR4 was defective in patients with UC, suggesting an over-expression of molecules that participate in leukocyte activation and recognition [18].

Accordingly, Paiva *et al.* (2011) evaluated the inflammatory activity in endoscopically normal ileal pouch mucosa and found a higher TLR4 expression in UC when compared with FAP patients and control individuals. These results suggest that pouchitis may be the consequence of positive regulation of intracellular pathways activated by bacterial products, which may contribute to the maintenance of an inflammatory-prone state in UC patients [19]. Malfunctioning TLR signaling can lead to inflammatory disorders, by NF- κ B (nuclear transcription factor κ B) activation, which plays a role in the transcription of several genes responsible for controlling the innate response, such as interleukin (IL)-1, IL-2, IL-6, IL-12, and tumor necrosis factor (TNF)- α . Several studies have demonstrated the presence of pro-inflammatory cytokines in ileal pouches of UC patients, such as TNF- α , IL-1 β , IL-6, IL-8, and interferon (IFN)- γ [8, 20-22]. A similar cytokine pattern was shown through the isolation of mononuclear cells from the *lamina propria* and culture of samples from colonic mucosa of UC patients and ileal mucosa from ileal pouch of UC patients who underwent this procedure. Pouchitis in UC is also characterized by a decreased expression of IL-10. These findings suggest that the inflamed reservoir may reactivate the immunological mechanisms that lead to UC [19-25].

Higher levels of TNF- α , IL-1 β , IL-6, and IL-8 were verified in the ileal pouch mucosa of UC patients when compared with FAP. Moreover, both UC and FAP patients had higher levels of TNF- α when compared with the control group ($p < 0.05$). However, there was no difference in NF κ B activation among these groups [26]. Signal trans-

Pouchitis and the current molecular mechanisms

Table 1. Current molecular and clinical aspects involved in the pathogenesis of primary pouchitis

Factors	Authors	Comments
Immune alterations	Segal et al., 2010. [18]	-Over-expression of molecules that participate in leukocyte activation and recognition
	Kusunoki et al., 2006; Leal et al., 2011. [17, 19]	-TLR2 and TLR4 expression upregulated
	Koltun et al., 2016; Barbara et al., 1994; Keighley et al., 1995; Goes et al., 2008. [129, 20, 21, 26]	-Presence of pro-inflammatory cytokines in ileal pouches of UC patients, such as TNF- α , IL-1 β , IL-6, IL-8, and interferon (IFN)- γ might explain higher rates of pouchitis in this group
	Desreumaux et al., 2000. [22]	-Decreased expression of IL-10
	Neurath, et al., 2005. [28]	-Increased activation of the pro-inflammatory transcription factor STAT1
	Herzig, et al., 2006. [31]	-Decreased levels of α and β defensins
	Kusunoki et al., 2020. [129]	-Increased IFN- γ mRNA expressions in patients who developed pouchitis
	Ponsioen et al., 2021. [130]	-Increased MAdCAM-1 expression in active inflammation in the pouch
Genetic susceptibility	Peña et al., 2005. [35]	-Single-nucleotide polymorphisms (SNPs) involved in the susceptibility to pouchitis and its severity
	Koltun et al., 2012. [34]	-Mutations in the nucleotide-binding and oligomerization domain (NOD) and TNFSF15
	Peña et al., 2005. [35]	-Association of TLR9-1237C and CD14-120T alleles with the development of chronic pouchitis
Autophagy/Apoptosis	Leal et al., 2018. [64]	-Modulation of macroautophagy markers leading to the mucosal inflammation with an increase of p62 in the ileal pouch
	Heriot et al., 2006. [62]	-Differential expression of Beclin1 in the colon of UC patients
	Shen et al., 2012. [134]	-Increase in the deep crypt apoptosis in autoimmune pouchitis
	Góes, et al., 2008. [26]	-Increased expression of anti-apoptotic protein (phospho-BAD) in UC patients could explain higher rates of pouchitis in this group
Mucosal ischemia-reperfusion	De Simone et al., 2001. [47]	-Increase of iNOS activity levels in the inflamed pouch compared with uninfamed control pouches
	Blikslager et al., 2017. [113]	-TLR4-TRAF6 pathway and the effects of SOCS-1 may participate in the regulation of multi-organ damage caused by intestinal ischemia-reperfusion injury
Environmental and clinical factors	Thirlby et al., 2000. [66]	-Greater occurrence of pouchitis in patients with extensive UC
	van Heerden et al., 1990; Nozawa et al., 2019; Targan et al., 2007. [67, 131, 78]	-Pouchitis frequently occurred with a higher risk in patients with extraintestinal manifestations
	D'Hoore et al., 2008. [79]	-Younger age represents a higher risk of developing pouchitis
	Coates et al., 2018. [132]	-Cessation of smoking was associated with an increase in the development of pouchitis
	Pardi et al., 2013. [133]	-ANCA-positive patients present a higher risk of developing chronic pouchitis after IPAA
	Fukushima et al., 2012; Petrovska et al., 2010; Dotan et al., 2015. [89, 93, 94]	-The development of pouchitis was associated to a decrease in bacterial diversity in the microbiota of the ileal pouch, which would influence adequate functional performance

ducer and activator of transcription (STAT) also play a role in IBD pathophysiology, mainly in UC. Higher activation and expression of STAT1 have already been identified in UC mucosa, whereas in Crohn's disease (CD) this pathway is not activated. High levels of SOCS-3 (suppressor of cytokine signaling), an inhibitor of STAT activation, were observed in CD patients and normal controls [27-29]. Interestingly, UC patients with non-inflamed ileal pouches after proctocolectomy had higher STAT1 levels when compared to the control group and FAP. These same patients also had higher levels of IFN- γ when compared with controls [30]. Patients with pouchitis also presented the same pattern, showing an increased activation of the pro-inflammatory transcription factor STAT1 in mucosal biopsies from inflamed pouches compared with both uninflamed and normal preoperative ileum [28].

The mechanisms of the innate immune response are essential for primary response in the intestinal mucosa against antigens. Defensins are important components in the innate immune response and play a key role in the homeostasis of the intestinal microbiota [24, 31]. Kiehne *et al.* (2006) found decreased levels of α and β defensin in UC pouchitis when compared with FAP. On the contrary, a low incidence of pouchitis in FAP was correlated with increased levels of hBD-1- β -defensin and low levels of inflammatory cytokines [31].

Genetic polymorphisms associated with pouchitis

Genetic technological advances using genome-wide association study (GWAS) have enabled researchers to identify the correlation between the genome and the occurrence of IBD, providing valuable information for the scientific community. Specific gene signatures can predict pouchitis and likely postoperative complications [32-34].

Andus *et al.* (1997) found an association between the IL-1RN*2 allele (IL-1ra allele 2 gene) and the reduction of IL-1ra levels in colon mucosa of patients with IBD. This genetic variant can prevent adequate control of inflammation in the intestinal mucosa and it is associated with the risk of developing pouchitis in UC patients [32]. This association was not con-

firmed by the study carried out by Aisenberg *et al.* (2004) [33].

Lammers *et al.* (2005) aimed to investigate the single-nucleotide polymorphisms (SNPs) involved in the susceptibility to pouchitis and its severity. Based on the analyses of 157 IPAA patients, they found an association of TLR9-1237C and CD14-120T alleles with the development of chronic pouchitis [35].

In this context, Sehgal *et al.* (2012) analyzed SNPs associated with pouchitis after IPAA in patients who developed severe pouchitis for at least 2 years after surgery. The results demonstrated that mutations in the nucleotide-binding and oligomerization domain (NOD) and TNFSF15 locus were correlated with severe cases of pouchitis [34].

In the same way as TLRs-type receptors, NOD2 (CARD15), one of the members of the NOD family, is associated with increased susceptibility to several inflammatory diseases, especially IBD. These studies have demonstrated a potential association of NOD variants (rs2066874, NOD2insC, 1007fsCins) with the development of pouchitis [36-38]. NOD2 is a cytoplasmic molecule involved in the detection of microbial cell wall components and the regulation of inflammatory processes, as well as apoptosis. It recognizes muramyl dipeptide (MD) from Gram-positive and Gram-negative bacteria that stimulate the enteric immune system. Thus, if there is a loss or reduction in NOD2 function, the immune response to bacterial pathogens may be altered [39, 40]. Through these studies, Meier *et al.* (2005) demonstrated that mutations in the NOD2 gene may predispose the development of pouchitis after IPAA in patients with UC. The percentage of NOD2 mutations was significantly higher in patients with pouchitis when compared to individuals without pouchitis (18% and 8%, respectively) [41]. In addition, a multicenter study that enrolled 714 patients, demonstrated that the risk variant NOD2insC was associated with chronic pouchitis with an added ratio of 3.2 when compared with those who did not have pouchitis [42].

Huang *et al.* (2017) performed the first longitudinal study that analyzed transcriptomic changes in the ileal pouch during the first year after IPAA. UC patients presented a shift in their transcriptional program after functionalization.

Pouchitis and the current molecular mechanisms

As a result of reprogramming, many of the genes expressed in the colon became active in the ileal pouch mucosa, while many specific genes in the ileal region became less expressed. This colon-like ileal shift can lead to increased susceptibility to the disease. Mainly, the authors found genetic factors that could explain the colon involvement and its consequent propensity to develop pouchitis in the ileal mucosa of these patients. These changes would then be related to colonic metaplasia, suppression of xenobiotic metabolism, increased immune activation, and P450 signaling pathway [43]. Huang's group confirmed what was seen by Morgan *et al.* (2015). The colonic marker CEACAM-7 was one of the main upregulated genes in the UC pouch [44]. The expression of matrix metalloproteinase (MMPs) in the ileal pouch is similar to that seen in inflamed tissues caused by IBD, with increased MMP1, MMP2, and MMP3 in both pouchitis and UC [45-50].

Several studies have analyzed the changes in gene expressions and, consequently, in histological characteristics that occur in both UC and FAP pouches. Paziewska *et al.* (2015) analyzed the gene array differences between UC and FAP pouches. The authors observed different gene expressions among pouches, suggesting that a UC pouch is more susceptible to developing pouchitis [51]. Similarly, Ben-Shachar *et al.* (2013) also utilized DNA microarrays to compare the normal terminal ileum of UC patients, healthy pouches, chronically inflamed pouches, and patients with Crohn's-like pouchitis. The analysis showed that patients with chronic pouchitis and Crohn's-like pouchitis presented several genetic changes, which are more severe when compared with individuals with normal ileum [52].

Autophagy and apoptosis pathways

Autophagy is responsible for the regulation of cellular processes that involve cell survival and death. When it occurs correctly, it promotes survival through the generation of energy for mitochondrial oxidation [53, 54]. However, defects or exacerbations in cellular autophagy processes have been associated with cellular death and the induction of stress signals [55]. The role of autophagy has already been demonstrated in IBD [56-61].

Lovegrove *et al.* (2006) found a differential expression of Beclin-1 protein, which is involved

in the autophagy initiation process, in the colon of UC patients [62]. Similarly, Leal *et al.* (2010) have demonstrated the correlation between signaling pathways and apoptosis in the ileal pouch mucosa of patients who underwent IPAA [63]. To determine transcriptional changes that occur in the inflammatory process of the ileal pouch, Paiva *et al.* (2018) evaluated the molecules involved in the autophagy pathway in the ileal pouch mucosa of FAP and UC patients. They found defective autophagy markers in both FAP and UC pouches, even in endoscopically normal mucosa, suggesting a mechanism for mucosa inflammation predisposition [64].

Another study suggests an important role for epithelial apoptosis mediated by Fas-Fas-L predisposing patients to pouchitis. Increased levels of epithelial apoptosis lead to increased villous atrophy when comparing UC patients with and without pouchitis [65]. Taken together, autophagy and apoptosis pathways may partially justify the differences in the molecular mechanisms of pouchitis in UC and FAP.

Environmental and clinical factors

In addition to immunological and genetic aspects, the occurrence of pouchitis after IPAA has been associated with environmental and clinical factors, such as length of UC, involvement of extraintestinal sites, active smoke, age during surgery, and serological markers.

Length of UC lesions: A study carried out by Simchuk *et al.* (2000) analyzed short- and long-term outcomes of patients who underwent IPAA for 12 years. This data demonstrated a greater occurrence of pouchitis in patients with extensive UC when compared to individuals with only left-sided involvement, which suggests a strong association between extensive UC and risk for pouchitis [66].

Extraintestinal involvement: Besides intestinal manifestations, there is often an IBD extraintestinal (EIM) occurrence, which can involve the joints, eyes, liver, and skin. Lohmuller *et al.*, (1990) analyzed the association between EIM and the development of pouchitis in 734 patients. The study suggests that pouchitis frequently occurred with a higher risk in patients with EIMs [67]. Primary Sclerosing Cholangitis (PSC) is one of the UC-related EIMs and it is also a chronic autoimmune hepatopathy, characterized by inflammation and fibrosis of intra-

and extrahepatic bile ducts. The association between PSC and pouchitis after IPAA has not been fully established. Some researchers investigated this context and suggested a negative correlation. However, other studies indicate that PSC could be a protective factor for CD in the pouch, given its association with inflammation in the afferent limb of the pouch [68, 69].

Another very common EIM in IBDs is arthritis, in which the prevalence rates are 14.3% to 44% among patients. Arthritis is a rheumatological autoimmune disease that affects the synovial membranes of multiple joints (hands, wrists, elbows, knees, ankles, feet, shoulders, cervical spine) [70]. As already discussed in the genetic section, mutations in the NOD2 allele are risk factors for the occurrence of pouchitis, but a mutation in this allele is also associated with auto-inflammatory diseases, like arthritis. Seril *et al.* (2016) described two cases of pouchitis after IPAA in UC patients that also presented EIM. In their study, both patients had mutations in the NOD2 allele and presented symptoms of the arthralgia spectrum (polyarthralgia and migratory oligoarthritis) [71].

Active smoking: A consensus on the consequences of smoking on UC and pouchitis has not been yet reached. Therefore, several studies have attempted to analyze the impact of smoking on predisposition to pouchitis. Initial experimental studies in rats suggested that active smoking reduced the risks of developing both UC and colitis [72, 73]. Interestingly, two studies have suggested a protective role for tobacco against pouchitis in active smokers [74, 75]. Based on these findings, the use of small doses of tobacco was used in several case reports as a treatment for a patient with severe disease [76, 77]. Furthermore, a study by Fleshner *et al.* (2007) found opposite results to those seen previously, in which active smoking increased the incidence of acute pouchitis [78].

Age as a risk factor: The literature suggests that the age of UC diagnosis or IPAA performance is associated with the risk of developing pouchitis. In this context, Ferrante *et al.* (2008) analyzed for 6.5 years 173 patients with UC who underwent IPAA. Their results showed that undergoing surgery at a younger age represents a higher risk of developing pouchitis [79].

Similarly, Uchino *et al.* (2013) showed, in a Japanese cohort, that 44.3% of 149 patients with UC who underwent IPAA developed pouchitis. Of these, the mean age was lower in those who developed pouchitis. Differently from Ferrante's *study*, Uchino showed that individuals younger than 26 years had a higher risk of developing chronic pouchitis when compared with patients older than 26 years [80].

Microbial changes and pouchitis

Alterations in luminal microbiota are now regarded as a key element for the development, evolution, and treatment of pouchitis [81]. The use of antibiotics (such as ciprofloxacin, rifaximin, or metronidazole) and probiotics may help control the symptoms and decrease inflammation in many patients with IPAA [8]. Besides, increased serum levels of antibodies against bacterial antigens have been observed in patients with an inflamed pouch. This evidence provides further support to the hypothesis of a relationship between pouchitis and an imbalance of bacterial overgrowth [82].

In addition, several studies compared the intestinal microbiota of UC and FAP patients undergoing IPAA in order to understand the mechanisms that contribute to the development of pouchitis. Tyler *et al.* (2013) compared the microbiota of patients with FAP and UC at different stages of IPAA. In the first stage of an ileoanal pouch operation (total colectomy), UC patients showed less bacterial diversity in mucosa samples in the colon before colectomy compared to FAP patients presenting lower levels of *Clostridium perfringens*. In addition, microorganism species, such as *Klebsiella* and *Lactobacillus* that are normally found in the formed pouch, were not present in pre-colectomy [83, 84].

Almeida *et al.* (2008) also associated the predominant presence of *Veillonella* species in the mucus of the terminal ileum with a persistently abnormal intestinal microbiota in UC patients [85]. Morgan *et al.* (2015) associated the presence of *R. gnavus*, *B. vulgatus*, and *C. perfringens* genera and the absence of *Blautia* and *Roseburia* genera with the predisposition to develop pouchitis [44]. Fecal samples of UC patients had a greater predominance of anaerobic and decreased aerobic bacteria in the ileal pouch when compared with FAP patients [84].

Microbiota and longitudinal follow-up post-surgery IPAA: Changes in microbiome diversity are strongly related to the development of pouchitis in the first-year post-surgery [85-97]. A great evidence of change in microbial constitution immediately after the ileostomy closure and the restitution of fecal flow through the pouch has already been observed [92]. Hinata *et al.* (2012) noticed changes in microorganism patterns two months after the restoration of intestinal continuity, with an increase in colon-predominant anaerobic bacteria in fecal samples with a decrease in ileum predominant species [89]. When compared to control groups, Almeida *et al.* (2008) found an increase of *Enterobacter sp* and *Klebsiella sp* and a decrease of *Enterococcus sp* and *Fusobacterium sp* in the ileal pouch [91].

Maharkashak *et al.* (2017) investigated for one year after surgery the profile of the microbiota in UC patients. They found a significant decrease in the diversity of microorganisms in those with pouchitis in less than one year when compared to those who did not develop inflammation in the first year. Interestingly, when patients were followed up for 3 years after surgery, there was an increase in microbial diversity, suggesting that microbial diversity may indicate a predictor of local inflammation [87].

Fungal dysbiosis and pouchitis: In a normal pouch, a great diversity of fungi coexists with bacteria in similar intestine niches, being represented mainly by *Aspergillus*, *Candida*, *Nigrospora*, and *Rhodotorila* [91]. Although fungal variation in pouchitis has been less explored, a recent study confirmed the role of fungal dysbiosis induced by 0.5% Fluconazole in experimental pouchitis and by 5% dextran sulfate sodium for 7 consecutive days in a rat model of IPAA, showing that dysbiosis increased mortality, weight loss and worsened CD4+ cell infiltration and severity of pouchitis [91].

The functional importance of the microbiota and the impact on the development of pouchitis: Gut microbiota is essential for several host physiological processes, including digestion of dietary factors, protection against colonization of pathogenic microorganisms, and development and performance of the gut immune system [92]. The development of pouchitis has been linked to a decrease in bacterial diversity in the microbiota of the ileal pouch, which

would influence adequate functional performance [89, 93, 94].

Ruminococcus: (Ruminococcaceae Family), for example, is important for the degradation of polysaccharides in the intestine that are essential for the maintenance of mucosal integrity and other benefits for the host [95, 97], and a significant increase in *Ruminococcus* was found in the normal ileal pouch when compared to patients with pouchitis [87].

Bacteroidetes and Firmicutes are the main phyla in the gut (about 90%) and they consist of relevant bacteria in the production of short-chain-acid (SCFA) metabolites. SCFA substrates are important sources of energy for colonic epithelial cells, they also present anti-inflammatory properties, and maintain epithelial barrier integrity [97]. SCFA is the main product of fermentation of dietary carbohydrates and fiber by mandatory anaerobic bacteria. The reduction of SCFAs has been found in cases of pouchitis when compared to an uninfamed ileal pouch [98-100].

Butyrate is an important SCFA that contributes to gut health and plays an important role in maintaining the epithelial barrier function, reducing oxidative stress, improving the immune system, and inducing regulatory T cells [101-104]. Examples of butyrogenic bacteria are Gram-positive Firmicutes belonging to *Clostridium* clusters XIVa and IV, standing out the species *F. Prausnitzii*, *Roseburia spp*, and *Eunacterium rectale* [105].

Enterocytes utilize butyrate and glutamine as fuel. However, a significant decrease in glutamine oxidation was observed in the ileal pouch mucosa while butyrate oxidation remained constant [104, 106]. Thus, the presence of butyrogenic cells was considered beneficial for the ileal pouch offering high concentrations of butyrate even with changes in microbial composition [103]. In UC patients, when compared to non-UC controls, a decrease of butyrate oxidation in the ileum is observed. That could indicate a predisposition to reduce butyrate metabolism when the ileal pouch is performed [106]. Corroborating this hypothesis, a decrease of three bacterial groups, which belonged to the Clostridiales order, was found in the pre-pouch ileum. These bacteria are butyrate-producing and are considered beneficial for a healthy gut

[86]. Patients who develop a healthy microbiota after IPAA may have the development of specific butyrate-producing community bacterial profiles, similar to those found in a healthy colon [107].

A higher incidence of sulfate-reducing bacteria in UC pouches compared with FAP pouches may be attributed to the low incidence of pouchitis in FAP patients. This bacterium uses sulfur instead of oxygen for breathing, leading to the production of hydrogen sulfide, which, in turn, inhibits butyrate oxidation and thus reduces the concentration available as nutrition to the intestinal epithelial cells, resulting in damage to the ileal pouch mucosa [86, 108].

Colon-like metaplasia of ileal pouch: The colonic microbiota is efficient for the degradation of complex indigestible carbohydrates. On the other hand, the small intestine can metabolize small carbohydrates and adapt rapidly to the fluctuation of nutrient availability in the lumen [109-111].

Even though the pouch originates from the ileum, the pouch bacterial community changes, becoming more similar to the colon [85, 88, 89]. Fecal stasis may cause greater bacterial load and increase the adhesion of Bacteroidetes and Firmicutes [109-112]. An increase in facultative anaerobic bacteria and a decrease in anaerobes occur after ileostomy closure, becoming similar to the colon's microbiota [84, 88]. Almeida *et al.* (2008) described that the decrease of typical small bowel microorganisms, such as *Enterococcus spp* and *Lactobacillus spp*, occurs in less than 2 months, contributing to a similar colonic microbiota [85].

One of the reasons why fecal stasis in the ileal pouch has been implicated in colon metaplasia and mucin glycosylation transformation is the fact that such changes do not occur in ileostomies before IPAA [110, 111]. Another factor that contributes to this bacterial alteration is the adaptation of the pouch due to the loss of glutamine oxidation based on butyrate oxidation, which also resembles the colon and contributes to the development of colonic metaplasia [104]. Even with evidence of colonic-like composition in the ileal pouch, the clinical significance of such aspect remains unclear. A hypothetical explanation for this is that the development of pouchitis would be related to

the failure of the ileal pouch to develop a mature bacterial community similar to that found in the healthy colon [90].

Limitations and bias: The study of microbiota and pouchitis has expanded in the last few decades, but it still presents many obstacles. Most of the data come from North American and European countries, with a much smaller number of studies coming from Africa, South America, and Asia, which can lead to important biases [107]. For example, differences among diets can alter the host's intestinal microbial composition [85].

Another limitation is the temporal dynamics of the composition of the small intestine microbiota, which may change in the morning and afternoon profiles over 9-18 days. Furthermore, certain drugs may influence microbiota, such as antibiotics, proton pump inhibitors, among other medications [87, 110, 111].

Finally, the heterogeneity of analysis techniques and sampling strategies are also factors that hinder conclusions about the relationship between intestinal microbiota and pouchitis. Moreover, we still do not know whether changes in the intestinal microbiota are the causes or the consequences of changes in the immune system or genetic factors.

Mucosal ischemia-reperfusion and oxygen radicals in ischemia-induced lesions

Intestinal ischemia-reperfusion occurs in a variety of pathophysiological situations. This complication is observed in patients with acute mesenteric ischemia, severe blood loss, and/or hypovolemia, frequently observed in patients undergoing major surgery or in patients with trauma, shock, or sepsis. Besides that, intestinal ischemia-reperfusion may be involved in the development and perpetuation of intestinal inflammation, including in UC and FAP patients who undergo IPAA [4, 6].

At a cellular level, intestinal ischemic injury reduces cellular mitochondrial ATP (adenosine triphosphate) generation, activates hydrolases, reduces cell membrane selective permeability, and increases calcium influx into ischemic cells. Reperfusion may exacerbate the extent of injury through the activation of an intense systemic inflammatory response, such as

marked proinflammatory cytokine release, production of reactive oxygen species (ROS), increased expression of nitric oxide (NO), TLR-4 signaling, and activation of inflammatory transcription factors, among other pro-inflammatory mechanisms [4, 8, 10, 11].

Nitric oxide has been suggested to play an important role in the physiology and pathogenesis of the gastrointestinal (GI) tract. Indeed, nitric oxide production by the inducible nitric oxide synthase (iNOS) may act as a protective agent at the onset of the inflammatory response in the GI tract. Recent studies have suggested that the overproduction of NO by iNOS is detrimental during chronic inflammation. In this regard, many investigators have shown that IBD is associated with increased mucosal production of NO and increased iNOS expression [4, 8, 10, 11, 47]. Similar results were found by Ulisse *et al.* (2001). They observed a significant increase of iNOS activity levels in the inflamed pouch compared with uninflamed control pouches [47].

In addition, studies involving SOCS-1 and the pro-inflammatory TLR4-TRAF6 signaling pathway have suggested that intestinal ischemia-reperfusion injury affects other vital organs, such as lung, liver, and kidney, through amplification of organ-specific inflammatory stimuli to systemic inflammatory responses. These studies indicate that the TLR4-TRAF6 pathway and the effects of SOCS-1 may participate in the regulation of multi-organ damage caused by intestinal ischemia-reperfusion injury [113].

In this way, although many studies have provided essential information regarding the mechanisms of inflammation and apoptosis regulation during intestinal ischemia-reperfusion injury, further studies are needed to fully understand the relationship between the iNOS activity, TLR4-TRAF6 pathway, and SOCS-1, especially in patients with IBD and in those with pouchitis. Acute ischemia is a topic that requires a lot of attention from the surgeon who treats IBD, mainly because it is a potentially fatal clinical emergency, as well as difficult to manage clinically with consequent high morbidity and mortality. Moreover, when the ischemia is chronic, it can be a silent process that may lead to pouch dysfunction and even complete loss of the reservoir [8, 10, 11, 110-113].

Serological markers of developing pouchitis

The measurement of direct antibodies against microbial antigens is often used to distinguish CD and UC. In addition to the diagnostic factors, these antibodies enable us to predict possible complications, responses to medications, and the need for surgical intervention [114-116].

Among the identified antibodies are: Perinuclear anti-neutrophil cytoplasmic antibody (pANCA) and the anti-*Saccharomyces cerevisiae* antibody (ASCA); the first is usually identified in 41-73% of UC patients and 6-38% in CD patients, and the latter is more specific for CD [114].

Antibody titers are associated with the development of pouchitis. However, a definite consensus is still lacking. Fleshner *et al.* (2001) aimed to evaluate whether pANCA can be related to acute and/or chronic pouchitis after IPAA. Sixty-three percent of the patients enrolled in the study expressed levels of pANCA. After a 32-month follow-up, 34% of those patients developed pouchitis. In addition to these findings, the cumulative risk of developing chronic pouchitis in patients with high pANCA expression was significantly higher than in those with lower antibody expression [115].

These findings were confirmed by Tyler *et al.*, who studied endoscopic data from 399 patients with UC who underwent IPAA at Mount Sinai Hospital in Toronto, Canada. More than 16% of the patients enrolled developed chronic pouchitis, and 12.5% developed pouchitis and Crohn's disease Like (CDL), of which 14% were positive for ASCA. These results demonstrated an association between pANCA and inflammatory complications in the pouch, in addition to an association between the CDL phenotype and ASCA expression [83].

Contrarily, some studies have found no association between the presence of antibodies and the occurrence of pouchitis. Yasuda *et al.* did not find any association of pANCA and UC with pouchitis after IPAA. They concluded that antibody titer should not be used as a pre-surgery marker for predicting pouchitis development in the follow-up [116].

Reumaux *et al.* found similar data results by analyzing a group with 108 patients, 98 of the

patients underwent IPAA (88% with pouchitis, and 12% without). pANCA was determined in those individuals by immunofluorescence assay. The data demonstrated that 52% of the IPAA-patients without pouchitis and 67% of the patients with pouchitis were pANCA positive, concluding that there was no correlation between the serological marker and the disease [117]. Aisenberg *et al.* (2004), who analyzed a cohort of 102 patients with UC that underwent IPAA, performed another study that confirms this scenario. In the analyzed group, both pANCA and ASCA showed no correlation with pouchitis [41].

Therefore, the relationship between serological markers pANCA and ASCA and the probability of developing pouchitis after IPAA remains questionable.

Crohn's disease of the ileoanal pouch

Crohn's disease (CD) in the pouch after IPAA is an increasingly noticed diagnosis and it must be distinguished from the primary pouchitis we had mentioned in the sections above. Its etiology is not yet completely understood. On the one hand, CD may have been misdiagnosed before surgery, or, on the other hand, it may be a *de novo* CD. The cause of *de novo* CD in the ileoanal pouch remains largely unknown and has been related to the factors described above, such as a change in microbiome following pouch stasis, environmental factors, genetics, and others. Clinically, these patients present abundant symptoms and should be examined carefully when they develop fistulas, strictures, and chronic refractory pouchitis, as well as granulomas in the histopathological study. The diagnosis is sometimes challenging and takes into account clinical, radiological, endoscopic findings, and personal history. Patients can develop CD at any time after IPAA. Higher rates of pouch failure and the need for pouch excision have been observed [4, 118, 119].

Clinical management

Microbial-based therapies for pouchitis

With the growing evidence of the impact of dysbiosis on the development of pouchitis, different therapeutic options have also emerged which aim to manipulate microbiota.

Antibiotics

The effectiveness of antibiotics as a treatment in most pouchitis cases (metronidazole, rifaximin, and ciprofloxacin) is an important indication that dysbiosis plays a role in the pathogenesis of the disease. Biopsies from pouchitis patients have shown that antibiotic therapy reduces groups of specific bacteria including Bacteroides, Firmicutes, and Tenericutes [44]. While analyzing fecal samples from sick patients who were using antibiotics, they found a greater number of Firmicutes and lesser Proteobacteria when compared to those who were not taking antibiotics [120]. Ciprofloxacin has been linked to decreasing *Clostridium perfringens* and *Escherichia coli* and had less effect on anaerobic bacteria. Metronidazole reduced *C. perfringens* and anaerobic bacteria, but not *Escherichia Coli*. Thus, ciprofloxacin appears to be less harmful to non-pathogenic species and more efficient against pathogenic species than metronidazole [121].

Antibiotic treatment has also been linked to restoring SCFA concentrations to normal levels in cases of pouchitis [122]. Even though it is a widely used treatment, the risk of bacterial resistance and uncertainty of toxicity limits its long-term use.

Several other antibiotics have been reported to be effective in uncontrolled series of patients with pouchitis. Most patients with pouchitis are likely to have symptomatic improvement after 1-2 days of therapy with metronidazole or ciprofloxacin [121-123].

Probiotics and prebiotics

The use of probiotics has shown significant induction of remission and prevention of pouchitis recurrence, and also shows effectiveness after antibiotic-induced remission. The most widely used is VSL # 3, which has been shown to increase bacterial diversity within the ileoanal pouch in addition to decreasing fungal diversity when compared to placebo treatment, which restores the balance between fungi and bacteria [124].

VSL # 3 was associated with increased concentration of *Lactobacilli spp.*, *Bifidobacterium spp.*, and *E. coli* in biopsy samples. However, the

microbiota alteration after the use of probiotics was not long-term sustained [94, 95]. For pouchitis, probiotics have been used as a therapeutic option in cases of remission, but no data support the use of probiotics as primary therapy [124].

Welters *et al.* (2002) analyzed the effect of insulin supplementation for two weeks and found significant clinical and histological improvement associated with an increase in butyrate levels and a reduction in pH, *B. fragilis*, and also an increase in bile acid levels [125]. However, the same protocol had been performed by Meijer HP *et al.* (2000) without beneficial results [126].

Fecal Microbial Transplantation (FMT)

FMT treatment for pouchitis still shows controversial results as the studies show low clinical remission rates (9-21.5%). Research is still very limited due to the small study size and discordant protocols that include dose, frequency, donor selection, and route of administration of FMT as well as variation of each patient's microbiota [127].

Other therapies

Oral and rectal corticosteroids may be beneficial in active pouchitis. In patients who require oral steroids for induction of remission, maintenance therapy with immune modulators or low-dose oral budesonide should be considered. In cases of severe and refractory pouchitis, treatment with infliximab may be beneficial. Overall, infliximab appears to have good clinical effectiveness in selected patients achieving up to 80% short-term and around 50% long-term response [6, 10, 128]. Other therapies have been studied in pouchitis, including bismuth enemas, SCFA enemas, and glutamine suppositories, but the role of these treatments in pouchitis remains to be clarified [8].

Conclusion

This review correlated the development of pouchitis in UC patients with potential genetic, microbial, and immunological causes. Although the pathophysiology of pouchitis remains unknown, many relevant advances occurred in recent decades that allow physicians and researchers to develop better strategies to

treat pouchitis in the follow-up after IPAA. After the diagnosis of pouchitis, the patient is usually treated with antibiotics, suggesting that the disease is at least partially mediated by a bacterial component. Increases in the bacterial populations that are commonly considered pathogenic are seen in the uninfamed UC pouch compared with FAP pouches. Therefore, more longitudinal studies in the same patients after IPAA are necessary to evaluate how these multiple factors can influence the gut and pouch microbiota.

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Disclosure of conflict of interest

None.

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