The bacterial pathogenesis and treatment of pouchitis

S. D. McLaughlin, S. K. Clark, P. P. Tekkis, R. J. Nicholls and P. J. Ciclitira

Abstract: Restorative proctocolectomy (RPC) with ileal pouch-anal anastomosis is the opera-
tion of choice for patients with ulcerative colitis. Pouchitis is the most common cause of pouch
dysfunction. Although the pathogenesis of this disease is not well understood, bacteria have
been implicated in the disease process. Numerous bacterial studies have been reported over
the last 25 years with few unifying findings. In addition, many different treatments for pouchitis
have been reported with varying results. Antibiotic treatment remains the most studied and is
the mainstay of treatment. In this article we review the aetiology of pouchitis and the evi-
denced-based treatment options.

Keywords: dysbiosis, familial adenomatous polyposis, ileal pouch-anal anastomosis, inflam-
matory bowel disease, pouchitis, restorative proctocolectomy, ulcerative colitis

Background

Restorative proctocolectomy (RPC) with ileal
pouch-anal anastomosis is the operation of
choice for patients with ulcerative colitis (UC)
who require surgery and is also performed
in selected patients with familial adenomatous
polyposis (FAP). After surgery most patients
experience a satisfactory functional outcome.
Inflammation of the ileoanal reservoir (pouchitis)
is the most common long-term complication.
Pouchitis is diagnosed when histologically
proven acute inflammation is associated with
symptoms of frequency, urgency and liquid
stool in the presence of endoscopic evidence of
inflammation [McLaughlin *et al.* 2008b]. The
incidence of pouchitis is markedly different
between patients with UC and FAP. In UC the
quoted incidence varies from 20% to 50%
between studies [Hahnloser *et al.* 2007;
Romanos *et al.* 1997; Stahlberg *et al.* 1996]. In
FAP the incidence of pouchitis is much lower
than in UC and varies from 0% to 11% [Barton
et al. 2001; Nyam *et al.* 1997; Kartheuser *et al.*
1996; Fazio *et al.* 1995; Dozois *et al.* 1989]. This
large interdisease variation in incidence is
explained by the differences in definition and
length of follow up. Some studies have based
the diagnosis on symptoms alone whereas
others correctly have required a triad of symp-
toms, endoscopic findings and compatible histol-
ogy. Since the risk of pouchitis increases over

time, studies of a longer duration are more
likely to demonstrate a higher incidence of
pouchitis.

About 39% of patients who develop pouchitis will
have a single episode which is easily treated and
does not recur. The remaining 61% will suffer at
least one further episode [Lohmuller *et al.* 1990]
and about 5–19% of patients will develop
chronic relapsing pouchitis [Madiba and
Bartolo, 2001; Mowschenson *et al.* 2000; Hurst
et al. 1998]. In this article we review the aetiology
of pouchitis and the evidenced-based treatment
options.

The aetiology of pouchitis

Pathological similarities to ulcerative colitis and Crohn's disease

Similarly to UC and Crohn's disease (CD) the
mucosal inflammation that occurs in pouchitis
is localized to the areas with the highest concen-
tration of bacteria. There is a tenfold increased
risk of pouchitis in patients who undergo RPC for
UC compared with FAP. Therefore, it has been
suggested that pouchitis represents reactivation
of UC in the colonized small bowel of the pouch.

Prepouch ileitis (PPI; small intestinal inflamma-
tion proximal to the pouch inlet) is known to
occur in some RPC patients with UC [Slatter

Correspondence to:
**S. D. McLaughlin,
MBBS, MRCP**
Department of Biosurgery
and Surgical Technology,
Imperial College, Chelsea
and Westminster Hospital,
London SW10 9NH, UK
**simon.mclaughlin@
nhs.net**

S. K. Clark, MD, FRCS
Department of Surgery,
St Mark's Hospital,
London, UK

**P. P. Tekkis, MD, FRCS
R. J. Nicholls, MChir,
FRCS**
Department of Biosurgery
and Surgical Technology,
Imperial College,
London, UK

**P. J. Ciclitira, PhD, MD,
FRCP**
Department of
Gastroenterology,
Nutritional Sciences
Division, King's College,
London, UK

et al. 2008; Calabrese *et al.* 2007; Iwata *et al.* 2007; Kuisma *et al.* 2004]. The pathogenesis of PPI has not been studied. Ischaemia [Bell *et al.* 2006], CD and nonsteroidal anti-inflammatory drugs (NSAIDs) [Shen *et al.* 2006; Wolf *et al.* 2004] have been implicated in some cases, but where these factors have been excluded the pathogenesis is likely to be similar to that of pouchitis where bacteria are considered fundamental to the disease process. It is possible that reflux of pouch contents into the prepouch ileum analogous to backwash ileitis in UC, a higher bacterial load or more pathogenic bacteria may be responsible for the disease process. Evidence for this comes from knowledge that the antibiotic therapy is an effective treatment [McLaughlin *et al.* 2008a] and that PPI appears to be a risk factor for developing antibiotic resistance in patients treated with maintenance antibiotic therapy [McLaughlin *et al.* 2009b]. One group has suggested that inflammation proximal to the pouch is indicative of CD or associated with the use of NSAIDs [Shen *et al.* 2006; Wolf *et al.* 2004]. This study included 87 patients with available colectomy histology including 28 with UC, 32 with indeterminate colitis and 27 with CD. Only 5 UC patients had signs of PPI, all of whom were taking NSAIDs. Therefore, the study did not assess UC patients with PPI who were not taking NSAIDs. Recent work by our group has demonstrated that histologically PPI is a distinct entity which can be differentiated from CD [Bell *et al.* 2006] and it is therefore possible that PPI is similar to backwash ileitis seen in UC, providing evidence of similarity to UC.

Despite this there are several similarities between pouchitis and CD. Both are inflammatory conditions which can affect the small bowel, both respond to treatment with antibiotics and both improve with diversion of the faecal stream (importantly these treatments are not effective in UC). There is also evidence at an immunological level that pouchitis has a similar pathogenesis to CD. In UC inflammation is predominately Th2-cytokine driven, whereas in CD a Th1-mediated immune response dominates [Fiocchi, 1998; Gately *et al.* 1998; Sartor, 1991; Wolf *et al.* 1991; Kobayashi *et al.* 1989]. It might therefore be expected that pouchitis would be associated with a Th2-cytokine activation, but an increase in the Th1 cytokine interferon gamma (IFN-γ) has been described in acute and chronic pouchitis [Stallmach *et al.* 1998]. Our group has previously investigated the association between CD30

and pouchitis. CD30 was shown to be upregulated in UC but not in CD [Elewaut *et al.* 1998; Giacomelli *et al.* 1998]. In this study CD30 was elevated in acute pouchitis but not in those with chronic pouchitis [Thomas *et al.* 2001] and therefore it was postulated that this may be due to a more CD-type disease occurring in these individuals.

Evidence for pouchitis being a novel third form of inflammatory bowel disease
It has been suggested that pouchitis may represent a novel third form of inflammatory bowel disease (IBD) [Sandborn and Pardi, 2004]. The differences in treatment response seen in pouchitis patients compared with UC and CD could be considered to fit with this. Although antibiotics are of some clinical benefit in CD, they are much less so than in pouchitis where they are more effective than any other therapy. Interestingly there is no evidence that immunomodulators are effective in pouchitis (we return to this later) suggesting that the pathogenesis of the disease may differ from that of UC and CD where these agents are very effective.

The dysbiosis theory
An abnormal host–microbial interaction has long been implicated in all forms of IBD including pouchitis [Sartor, 2008]. There is evidence from clinical practice to implicate bacteria in pouchitis. In common with UC and CD, mucosal inflammation is localized to the area of gut with the highest concentration of bacteria [Sartor, 2008], antibiotics are effective treatment for both pouchitis and PPI [McLaughlin *et al.* 2008a; Shen *et al.* 2007a; Madden *et al.* 1994] and probiotics have been shown to reduce disease relapse [Mimura *et al.* 2004; Gionchetti *et al.* 2000], reduce the risk of disease onset [Gionchetti *et al.* 2005] and to be effective in mild to moderate pouchitis [Gionchetti *et al.* 2007a].

Based on the response of peripheral and local mononuclear cells our group has previously demonstrated that the inflammatory response in pouchitis appears to be at the local mucosal level and is not a general systemic reaction [Thomas *et al.* 2002] and that pouchitis-derived bacterial sonicates from metronidazole-sensitive bacterial species can stimulate healthy patients' mononuclear cells significantly more than corresponding sonicates from nonpouchitis patients [Bell *et al.* 2004]. Both studies providing indirect evidence

to implicate bacteria and justify studies of ileoanal pouch microbiota.

Studies of pouch microbiota

The interpretation of early work to establish the potential differences in bacterial microbiota between pouchitis and nonpouchitis patients is limited by the lack of universally accepted criteria for pouchitis prior to the Moskowitz score being described in 1986 [Moskowitz *et al.* 1986] and the pouch disease activity index (PDAI) in 1994 [Sandborn *et al.* 1994]. Many of these early studies were also limited by comparing well-functioning with poorly functioning pouches rather than nonpouchitis and pouchitis groups. In addition a further limitation of many studies was that many of the patients with a history of pouchitis were asymptomatic at the time of the study [Lim *et al.* 2006]. The majority of studies to establish the microbiota of the ileoanal pouch have been performed using culture methods, but it is now known that less than 50% of gut bacterial species can be cultured [Hayashi *et al.* 2002; Suau *et al.* 1999].

Estimates of the presence of 200−300 colonic species derived from culture-based studies have been revised to about 15, 000−36, 000 individual species following the introduction of 16s ribosomal RNA polymerase chain reaction (PCR) techniques [Frank *et al.* 2007]. There are several different PCR techniques each with a different sensitivity. Cloning and high-throughput sequencing of the 16 s gene is currently accepted as the 'gold standard' molecular method [Zoetendal *et al.* 2008; Lim *et al.* 2006] and therefore modern research should use molecular rather than culture-based techniques.

Studies using culture methods

Details of each of the culture studies are summarized in Tables 1 and 2.

Nicholls and colleagues studied 14 patients who had undergone RPC at least 6 months earlier, seven patients had inflammation of the reservoir and seven did not [Nicholls *et al.* 1981]. Inflammation of the reservoir was associated with a higher count of aerobic but not anaerobic bacteria.

Santavirta and colleagues studied faecal samples from 30 UC patients after RPC and 10 with a conventional ileostomy [Santavirta *et al.* 1991]. Nine had a history of pouchitis defined as increased stool frequency, bleeding and abdominal cramping, however only three of these patients were symptomatic at the time of the study and only two with a history of pouchitis had faecal samples analysed. The authors found no difference in the numbers of anaerobes and aerobes in those with a history of pouchitis compared with those who had no history of pouchitis but did report an increase in the total bacterial counts, numbers of anaerobes and ratio of anaerobes to aerobes compared with patients with a conventional ileostomy.

Ruseler-van Embden and colleagues studied faecal samples of 14 RPC patients (12 UC; 2 FAP) [Ruseler-van Embden *et al.* 1994]. Pouchitis was defined by clinical, endoscopic and histological criteria. Five were classified as having pouchitis and nine nonpouchitis. Of the former two were symptomatic when studied. There was no difference in the total numbers of bacteria between pouchitis and nonpouchitis patients but an increase in the number of aerobes was found. *Clostridium perfingens* was present in over 75% of the pouchitis and in less than 25% of the nonpouchitis samples. Bifidobacterium and Lactobacillus were found in only one pouchitis sample but were present in 84% of nonpouchitis samples.

Duffy and colleagues studied 25 patients (10 UC RPC, 7 FAP RPC, 8 ileostomy) none of whom had a previous history of pouchitis [Duffy *et al.* 2002]. In the UC RPC patients 80% of the samples contained sulphate-reducing bacteria (SRB) in contrast to none of the FAP or ileostomy samples. The authors found no significant difference between UC and FAP in counts of Lactobacilli, *C. perfingens*, Bacteriodes and Bifidobacterium or enterococci and coliforms.

Sandborn and colleagues cultured the stool from 30 patients (10 UC RPC non-pouchitis, 10 UC RPC pouchitis, 5 FAP RPC non-pouchitis and 5 ileostomy) [Sandborn *et al.* 1995]. Pouchitis was defined as a PDAI ≥ 7. The authors found a higher ratio of anaerobic Gram-negative rods in RPC compared with ileostomy patients and there were no differences between pouchitis and non-pouchitis samples in FAP and UC RPC.

A study was carried out by Ohge and colleagues of 50 patients including 9 UC RPC with previous pouchitis but no active disease for 1 year, 9 UC RPC with pouchitis in the last year, but inactive

Table 1. Studies comparing the microbiota of UC pouchitis with nonpouchitis patient samples.

Study	Patients	Faecal or mucosal study	Histological pouchitis?	Symptomatic pouchitis Y/N	Findings
Nicholls et al. [1981]	10 UC, 4 FAP	Faecal	Yes	No	Increase in aerobes in pouchitis
Santavirta et al. [1991]	30 UC-RPC (9 = Hx of pouchitis), 10 ileostomy	Faecal	No	Yes but only 3 were symptomatic	No difference
Ruseler-van Embden et al. [1994]	12 UC-RPC, 2 FAP-RPC	Faecal	Yes	Yes but only 2 of 5 were symptomatic	Increase in number of aerobes and *C. perfingens* in pouchitis
Sandborn et al. [1995]	10 UC-RPC nonpouchitis, 10 UC-RPC pouchitis, 5 FAP-RPC nonpouchitis, 5 ileostomy	Faecal	Yes	Yes (PDAI used)	No difference between pouchitis and nonpouchitis or FAP groups
Ohge et al. [2005]	18 UC-RPC previous pouchitis, 8 UC non pouchitis, 8 UC-RPC antibiotic treated	Faecal	No	None	Increased counts of SRB in patients with active pouchitis
Kuisma et al. [2003]	11 UC nonpouchitis, 21 with previous pouchitis	Faecal and mucosal	No	None	Increased aerobes and anaerobes in pouchitis faecal samples
Kmiot et al. [1993]	6 RPC pouchitis, 6 RPC nonpouchitis	Faecal	Yes	None	No difference
O'Connell et al. [1986]	14 nonpouchitis, 6 pouchitis	Faecal	No	Yes	No difference
Iwaya et al. [2006]	9 pouchitis, 13 non-pouchitis	Faecal	Yes	Yes (PDAI used)	Reduction in Bifidobacteria, Bacteriodaceae and Lactobacillus in the pouchitis group.

UC, ulcerative colitis; FAP, familial adenomatous polyposis; RPC, restorative proctocolectomy with ileal pouch-anal anastomosis; PDAI, pouch disease activity index.

Table 2. Studies comparing the microbiota of UC with FAP patient samples (no pouchitis group).

Study	Patient numbers	Faecal/mucosal	Histological Px?	Symptomatic pouchitis Y/N	Findings
Duffy *et al.* [2002]	10 UC-RPC, 7 FAP-RPC, 8 ileostomy	F		None	Increased SRB in UC patients
Sandborn *et al.* [1995]	10 UC-RPC nonpouchitis, 10 UC-RPC pouchitis, 5 FAP-RPC nonpouchitis, 5 ileostomy	Faecal	Yes	Yes (PDAI used)	No difference between pouchitis and nonpouchitis or FAP groups

UC, ulcerative colitis; FAP, familial adenomatous polyposis; RPC, restorative proctocolectomy with ileal pouch-anal anastomosis; PDAI, pouch disease activity index; SRB, sulphate-reducing bacteria.

for 6 weeks or more, 8 UC RPC more than 2 years ago and no previous pouchitis and 11 UC RPC pouchitis with ongoing antibiotic treatment [Ohge *et al.* 2005]. Stool samples were collected, and counts of SRB and output of hydrogen sulphide were measured. The authors found a fivefold greater release of hydrogen sulphide in all UC samples compared with the FAP samples except in the UC patients receiving antibiotic treatment, where there was no difference to the FAP group. Patients who had recently had pouchitis had a higher hydrogen sulphide production than those who had pouchitis more than 1 year previously which was higher than those who had never had pouchitis. SRB counts were higher in samples from active pouchitis patients than all other groups. Gosselink and colleagues cultured faecal samples from 13 patients with active pouchitis prior to antibiotic treatment, during treatment and following treatment. A comparison with nonpouchitis patients was not undertaken. They found that during an episode of pouchitis there was an increase in aerobes and decrease in anaerobes [Gosselink *et al.* 2004].

Kuisma and colleagues studied faecal microbiology and mucosal microbiology in 32 patients (11 nonpouchitis and 21 with a previous history of pouchitis) [Kuisma *et al.* 2003]. No patient had active pouchitis. The authors found a significantly increased total number of faecal anaerobic and aerobic bacteria in patients with a history of pouchitis as compared with nonpouchitis patients, but no difference in the bacteria from mucosal samples.

Kmiot and colleagues studied 46 UC patients (10 end ileostomy, 12 RPC pouchitis, 12 nonpouchitis good function and 12 nonpouchitis poor

function) [Kmiot *et al.* 1993]. Pouchitis was defined as endoscopic and histological inflammation and patients did not need to be symptomatic. Bacterial counts of stool samples were performed on 6 non-pouchitis and 6 pouchitis patients before and following metronidazole. The authors found no significant difference in the total aerobic or anaerobic bacterial counts between the non-pouchitis and pouchitis groups, there were also no differences between the numbers of *Escherichia coli*, *Streptococcus faecalis* or Bacteriodes in each group. Bacteriodes were not present in any samples following metronidazole. There was no significant difference in counts of *E. coli* or *S. faecalis* between the pouchitis and nonpouchitis groups following metronidazole.

O'Connell and colleagues studied 20 UC patients (8 nonpouchitis with good function, defined as <6 stools per 24 h, 6 nonpouchitis poor function, defined as >6 stools per 24 h, and 6 pouchitis, defined as episodic bloody diarrhoea and malaise) [O'Connell *et al.* 1986]. Three patients had active pouchitis and three did not. Jejunal aspirates and stool samples were analysed. There was no difference in the numbers of aerobic and anaerobic bacteria between the pouchitis and nonpouchitis groups in the jejunal aspirates and the values were within the normal range for healthy controls. Stool culture showed overgrowth of both aerobic and anaerobic bacteria in all samples compared with values for ileal chyme in health. There were no differences between the pouchitis and nonpouchitis groups. Iwaya and colleagues cultured stool samples from 22 UC RPC patients (9 pouchitis, 13 nonpouchitis) [Iwaya *et al.* 2006]. Pouchitis was diagnosed using the PDAI. The authors found a significant reduction in the

Bifidobacteria, Bacteriodaceae and Lactobacillus in the pouchitis group.

Studies using molecular methods

Falk and colleagues studied two UC RPC patients from the time of stoma closure for 1 year with repeated mucosal biopsies using terminal restriction fragment length polymorphism (T-RFLP), cloning and sequencing [Falk *et al.* 2007]. Neither patient developed pouchitis during this time. They found that the microbiota evolved during this time and differed between the two patients. The microbiotal composition was similar to normal colon except for the presence of *Clostridium perfringens* and Turicibacter. Kuhbacher and colleagues studied the differences in microbiota between patients with chronic pouchitis who were treated with the probiotic VSL#3 and those treated with a placebo following a successful remission induced by antibiotic treatment with ciprofloxacin and metronidazole [Kuhbacher *et al.* 2006]. This study did not compare nonpouchitis and pouchitis microbiota and did not study the microbiota of pouchitis patients before antibiotic therapy. The authors used real-time PCR, denaturing gel gradient electrophoresis (DGGE) and fluorescent in-situ hybridization (FISH) to study the microbiota. Patients who relapsed on placebo had a lower bacterial diversity and increased fungal diversity than those who maintained remission with VSL#3, with an increase in the diversity of Lactobacilli and Bifidobacteria species. However, the authors stated that the methods used did not reliably identify all bacteria present.

Casadesus and colleagues studied the possible role of Cytomegalovirus (CMV) in pouchitis; 34 UC RPC patients were studied [Casadesus *et al.* 2007]. Pouchitis was diagnosed using the modified PDAI [Shen *et al.* 2003] and Japanese classification of pouchitis (JCP) [Fukushima *et al.* 2007]. The JCP defines pouchitis as a condition with severe endoscopic findings or with two or more clinical symptoms and moderate endoscopic findings. Endoscopic biopsies from the pouch mucosa were taken on multiple occasions in each patient resulting in a total of 473 specimens (103 pouchitis patients, 370 nonpouchitis patients). CMV was detected in both pouchitis and nonpouchitis biopsies using PCR and sequencing and no significant difference was found in the incidence of CMV in each group when classified using the modified PDAI. CMV was, however, present in significantly more samples from pouchitis than from nonpouchitis patients when classified using the JCP. All pouchitis biopsies with CMV detected were obtained during the first episode of pouchitis, and in earlier samples from the same patients suggesting that CMV infection causes symptoms similar to pouchitis but is not the cause of pouchitis.

Four studies have used a molecular biological method to establish the differences between the microbiota in pouchitis and nonpouchitis patients. In the first Komanduri and colleagues studied ileal pouch biopsies and faecal samples from 33 patients (13 non-IBD patients, 5 UC pouchitis, 15 non-pouchitis) [Komanduri *et al.* 2007]. Pouchitis was classified using the PDAI. Ileal biopsies were taken from non-IBD patients who had an intact colon. Length heterogeneity PCR (LH-PCR) was performed and output data were expressed as electropherograms with the peaks representing different populations of microbiota with amplicons of different length in base pairs. Because this technique provides limited information about the bacterial differences at a species/phylotype level, the LH-PCR products from three non-IBD controls, five UC nonpouchitis and three UC pouchitis patients were pooled and cloned. Data were then filtered so that only phylotypes representing more than 5% of the total clone library were analysed. The authors reported an increase in the bacterial diversity in pouchitis samples compared with non-IBD controls, an increase in the proportion of Enterobacters and Fusobacter (proteobacteria), reduction in Streptococci (firmicutes) and a difference in the Rumicoccus species associated with pouchitis (*R. obeum*) and nonpouchitis (*R. gnavus*).

The use of pooled data for each of the disease groups was a significant source of error in this study. Since it is known that there is a wide variation in gut microbiota between individuals [Zoetendal *et al.* 2008; Komanduri *et al.* 2007], comparisons can only be made between groups when the data are derived from different individuals. In addition. according to the authors the technique used identified at most 73% of the microbial population.

The second molecular study (from our group) by Johnson and colleagues investigated the mucosal adherent microbiota in 32 RPC patients (7 UC pouchitis, 15 UC non-pouchitis, 9 FAP nonpouchitis, 1 FAP pouchitis) [Johnson *et al.* 2009].

Mucosal biopsies were first cultured on agar, the bacterial DNA was then extracted and amplified using PCR and profiles were generated using TRFLP [Johnson *et al.* 2009]. No differences between pouchitis and nonpouchitis groups or between FAP and UC groups were found but this study also had significant limitations. The use of an enrichment culture technique potentially favoured identification of bacteria supported by the culture conditions and TRFLP can only identify dominant species groups and is not able to identify individual bacterial species.

A further study by Lim and colleagues investigated the luminal microbiota in 20 RPC patients (5 UC pouchitis and 15 UC non-pouchitis) using TRFLP. They reported no differences in diversity or TRFLP profile between pouchitis and non-pouchitis samples. Again this study was limited by the use of TRFLP [Lim *et al.* 2009].

We recently performed a further study of the mucosal adherent microbiota in 24 RPC patients (8 UC pouchitis, 8 nonpouchitis, 5 FAP nonpouchitis and 3 FAP pouchitis). PCR amplification of bacterial 16S rRNA genes was performed and samples were individually cloned and sequenced to the species/phylotype level [McLaughlin *et al.* 2010]. There was a significant increase in Proteobacteria and a significant reduction in Bacteroidetes and *Faecalibacterium prausnitzii* in the total UC compared with the total FAP cohort with only small differences between the UC nonpouchitis and UC pouchitis groups and the FAP pouchitis and FAP nonpouchitis groups. Bacterial diversity was significantly greater in FAP nonpouchitis compared with UC nonpouchitis samples and significantly greater in UC nonpouchitis samples compared with UC pouchitis samples. No individual species or phylotype was specifically associated with either UC or FAP pouchitis.

Summary
The results from culture-based studies of pouch microbiota using culture methods are varied and inconclusive. Advances in our understanding of gut microbiota following the introduction of molecular techniques have led to the realization that culture-based techniques are inadequate for the study of gut microbiota. The differences in findings between earlier and the most recent molecular studies are likely to be explained by the different techniques employed and the limitations of the earlier studies. The findings of the most recent study [McLaughlin *et al.* 2010] suggest that a reduction in bacterial diversity but not dysbiosis occurs in pouchitis and that UC RPC patients have a different, less-diverse microbiota with increased Proteobacteria and reduced Bacteroidetes and *F. prausnitzii*.

Treatment of pouchitis
Before drug treatment is initiated a clinical diagnosis of pouchitis should be confirmed by flexible pouchoscopy and histological biopsy demonstrating acute inflammation since other causes of pouch dysfunction may present with similar symptoms [McLaughlin *et al.* 2008b]. In addition other precipitants should be considered; NSAIDs are associated with pouchitis and withdrawal may induce remission [Shen *et al.* 2007b]. Infectious agents such as *Clostridium difficile* and CMV should be excluded in those with pouchitis that fails to respond to antibiotics [Pfau and Lichtenstein, 2000].

Antibiotics
Ciprofloxacin or metronidazole for 14 days has been effective in several studies and should be considered as first-line treatment [Shen *et al.* 2001; Madden *et al.* 1994]. Topical metronidazole, given per anum, has also been shown to be effective and should be considered in patients intolerant to oral metronidazole who fail treatment with ciprofloxacin [Johnson *et al.* 2001]. Rifaxamin when used as a single agent for the treatment of pouchitis was found to be no more effective than placebo in a randomized placebo-controlled study of 18 patients [Isaacs *et al.* 2007].

Treatment options for patients who fail to respond to combination antibiotics or who relapse on maintenance treatment are very limited and some patients will have chosen indefinite diversion with an end ileostomy (with or without pouch excision). Using faecal coliform sensitivity testing our group recently demonstrated that ineffective combination antibiotic treatment may be due to the development of bacterial resistance. In a study of 15 patients who had failed to respond to empirical antibiotic treatment, ciprofloxacin resistance was identified in all stool samples and selection of another antibiotic based on bacterial sensitivity analysis resulted in remission in 80% of patients [McLaughlin *et al.* 2009a].

Other agents

Budesonide has been shown to be effective in those with refractory pouchitis who do not respond to a single antibiotic in an open-label study [Gionchetti *et al.* 2007b] but a randomized double-blind study found it to be no more effective than metronidazole [Sambuelli *et al.* 2002]. Its efficacy in those who fail to respond to ciprofloxacin and metronidazole or other combination regimes has not been demonstrated.

Bismuth-citrate carbomer enemas were shown to be effective in an open-label study using with 83% of patients entering remission [Gionchetti *et al.* 1997], however a subsequent double-blind randomised trial found no difference when compared with placebo [Tremaine *et al.* 1997].

There are no controlled trials of oral or topical mesalamine for pouchitis. A study which compared a combination of ciprofloxacin and tinidazole in pouchitis with a historical cohort treated with mesalamine found that 50% achieved clinical remission and 50% had a clinical response. Overall there was a significant reduction in the PDAI but no improvement in the Cleveland global quality of life (CGQoL) score, whereas of the patients treated with ciprofloxacin and tinidazole, 87.5% achieved a clinical remission and 87.5% achieved a clinical response with a significant reduction in PDAI and improvement in CGQoL score [Shen *et al.* 2007a]. Sulphasalazine was recently found to be effective for the treatment of pouchitis. In an open-label study of 11 patients 63% of patients were in remission after 8 weeks treatment [Belluzzi *et al.* 2010].

Allopurinol was found to be ineffective in preventing pouchitis in a randomised placebo controlled study [Joelsson *et al.* 2001]. Treatment with short chain fatty acids (SCFAs) was also ineffective in uncontrolled studies [de Silva *et al.* 1989].

Infliximab was beneficial in one study of patients with pouchitis and extensive PPI; however, they were found to have almost total small bowel inflammation (demonstrated by video-capsule endoscopy). It is possible therefore that this patient group had CD rather than UC [Calabrese *et al.* 2008]. In a recent retrospective uncontrolled study of 10 patients, seven maintained a clinical response at a median follow up of 8.5 months and endoscopy demonstrated four

to have continuing mucosal inflammation [Ferrante *et al.* 2010].

Other therapies that have been tried include oral prednisolone and azathioprine. There is, however, no good evidence that these are effective in pouchitis [Pardi and Sandborn, 2006]. There are two reports of the development of pouchitis in patients taking azathioprine for primary sclerosing cholangitis [Rowley *et al.* 1995; Zins *et al.* 1995] suggesting that immunomodulators may not be effective in pouchitis.

An algorithm for the treatment of pouchitis is given in Figure 1.

Treatment of prepouch ileitis

Two studies have reported the results of treatment for PPI. In the first, seven patients with chronic relapsing pouchitis and total small bowel inflammation identified at video-capsule endoscopy were treated with a standard infliximab induction regime of three infusions. Symptomatic and endoscopic resolution was reported in six [Calabrese *et al.* 2008]. Although CD had been excluded on histological grounds the presence of extensive small bowel inflammation raises the possibility that these patients were suffering from CD and not UC.

In the second study 14 patients with symptomatic PPI were treated with ciprofloxacin and metronidazole for 28 days. Twelve (86%) patients experienced symptomatic remission with a reduction in stool frequency from a median of 12 to 6 [McLaughlin *et al.* 2008a]. Given the cost and potentially significant complications associated with infliximab it is generally recommended that combination antibiotic therapy should be first-line treatment in patients with symptomatic PPI.

Maintenance therapy

It has been recommended that patients with chronic pouchitis who achieve remission following antibiotic therapy but relapse more than three times per year should be treated with maintenance therapy [Pardi and Sandborn, 2006]. Treatment with ciprofloxacin or the probiotic VSL#3 are recommended in the British Society of Gastroenterology IBD guidelines [Carter *et al.* 2004].

Studies using VSL#3. The probiotic VSL#3 was shown to maintain remission in 85% of patients with refractory pouchitis who had entered clinical
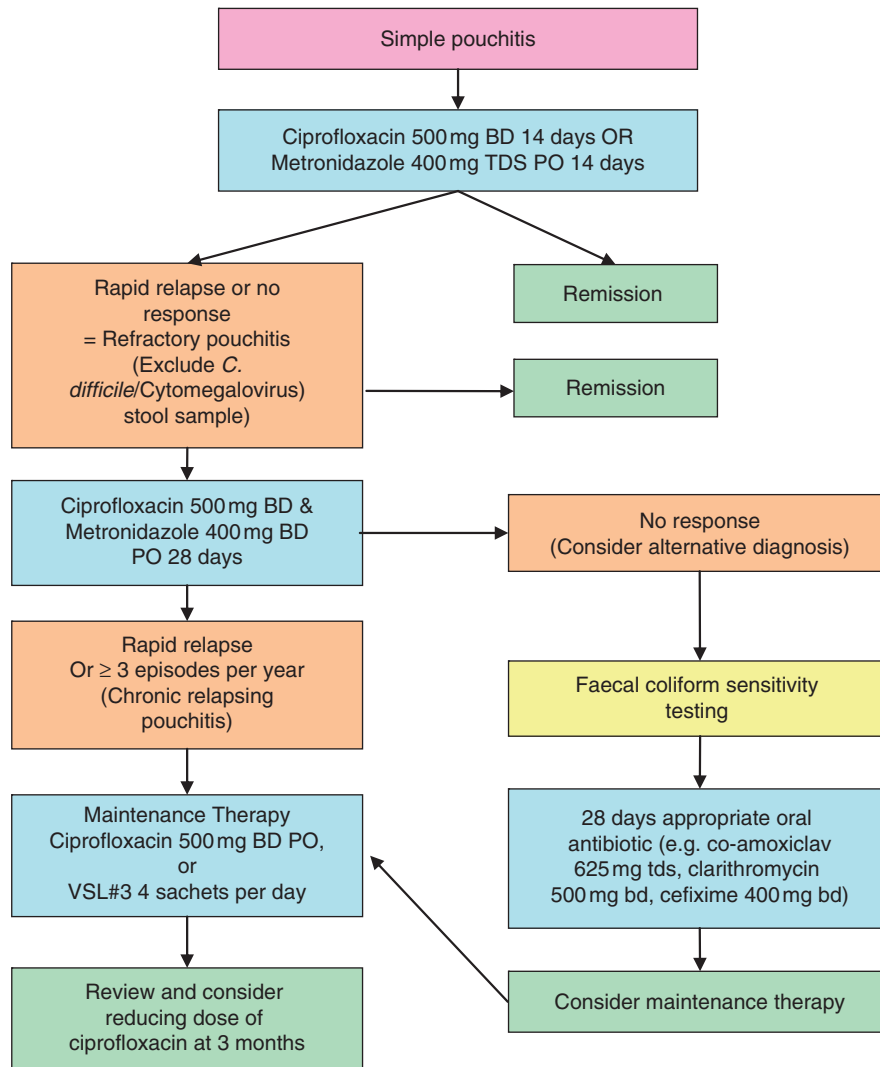
Figure 1. An algorithm for the treatment of pouchitis. Reproduced with permission from Blackwell Publishing Ltd (McLaughlin *et al.* [2008b]).

and endoscopic remission following 4 weeks treatment with ciprofloxacin and rifaxamin in a randomized placebo-controlled study of 40 patients [Gionchetti *et al.* 2000], whilst a later two-centre randomised placebo-controlled study of 36 patients found that 85% of patients could maintain remission following 4 weeks treatment with ciprofloxacin and metronidazole [Mimura *et al.* 2004]. In contrast, however, a more recent 8-month open-label clinical study reported that less than 20% of patients were able to maintain remission [Shen *et al.* 2005].

The reason for the difference in these results is not clear but differences in the design and protocol of the two studies may have contributed. The most important and modifiable factors were as follows.

Studies by Gionchetti and colleagues [Gionchetti *et al.* 2000] and Mimura and colleagues [Mimura *et al.* 2004] excluded patients who did not achieve complete or near-complete endoscopic remission with a score of ≤3 in the clinical component of the PDAI, whereas Shen *et al.* [2005] did not repeat the pouchoscopy after clinical remission. It is known that some patients do not achieve endoscopic remission despite clinical remission following antibiotic treatment [Madden *et al.* 1994] and it is possible that this subset of patients have a more difficult to treat disease which may not respond to probiotics.

Gionchetti's and Mimura's groups used a combination of ciprofloxacin and rifaxamin or metronidazole for 4 weeks whereas Shen and colleagues used a 2-week course of ciprofloxacin only to induce remission. It is possible that probiotic
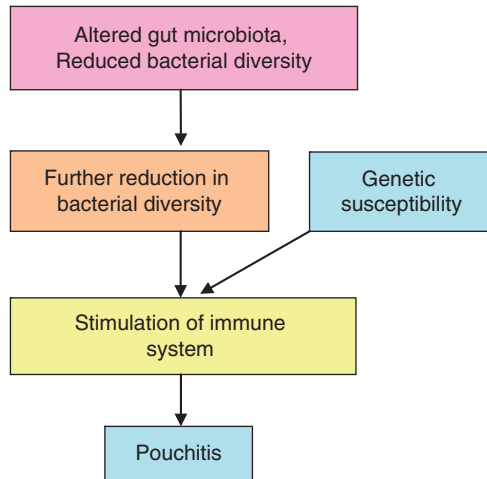
Figure 2. Potential mechanism of dysbiosis in pouchitis.

therapy is more effective following a combination of two different antibiotic agents for a prolonged period. Gionchetti and colleagues' and Mimura and colleagues' studies recruited patients with refractory pouchitis, defined as three or more episodes of pouchitis per year, whereas Shen and colleagues only recruited patients with chronic antibiotic-dependent pouchitis, defined as four or more episodes of pouchitis per year. Therefore, many of the patients included in the studies of Gionchetti's and Mimura's groups had less-aggressive disease in which maintenance of remission may have been easier to achieve.

Studies of antibiotic maintenance therapy. Two studies have reported the outcome of maintenance antibiotic treatment. In the first, 51 patients were treated with rifaximin maintenance therapy after induction of remission with single or combination antibiotic therapy [Shen *et al.* 2008]. At 3, 12 and 24 months 65%, 58% and 3% of patients were in remission. Importantly those who failed to achieve endoscopic as well as clinical remission were excluded from the study. In the second study (from our group) [McLaughlin *et al.* 2009b] which included both patients who had and who had not achieved endoscopic as well as clinical remission, retrospective data on 25 patients were reported. Several antibiotics had been used including ciprofloxacin, trimethoprim, coamoxiclav, colistin, nitrofurantoin, cefixime and trimethoprim. During a median follow up of 15.8 months no patient developed *C. difficile* colonization but 24% relapsed. In these, faecal coliform sensitivity

testing [McLaughlin *et al.* 2009a] demonstrated bacterial resistance in all cases and identified an appropriate change of antibiotic which resulted in the induction and maintenance of remission in all of these patients. Those who developed antibiotic resistance were successfully treated with two-weekly alternating antibiotics. In this study the presence of PPI but not failure of mucosal healing was associated with an increased risk of relapse.

The recent comprehensive molecular study comparing the microbiota in UC and FAP RPC patients [McLaughlin *et al.* 2010] identified similar differences to those reported previously in the colonic mucosa of UC and CD patients compared with patients without IBD. Frank and colleagues reported a reduction in Bacteroidetes and Lachnospiraceae and a reciprocal increase in Proteobacteria in a subset of IBD patients [Frank *et al.* 2007]. Other studies too have reported increases in Proteobacteria in IBD patients [Gophna *et al.* 2006; Seksik *et al.* 2003]. *F. prausnitzii* which was reduced in the UC RPC group has previously been postulated to have anti-inflammatory properties and found to be reduced in IBD patients [Sokol *et al.* 2009, 2008].

Conclusion

Bacterial diversity has been demonstrated in UC and CD and appears to be important in maintaining normal gut homeostasis [Andoh *et al.* 2007; Sokol *et al.* 2006; Ott *et al.* 2004]. In RPC there is a reduction in diversity in UC patients compared with FAP patients and a further reduction in diversity in patients with pouchitis [McLaughlin *et al.* 2010]. Overall these findings suggest that the pathogenesis of pouchitis is similar to that of UC and CD and that either a reduction in bacterial diversity leads to stimulation of the immune system resulting in mucosal inflammation or that dysbiosis predisposes UC patients to pouchitis by increasing the likelihood of immune system stimulation.

Antibiotics remain the mainstay of treatment for pouchitis and PPI. The majority of patients will enter clinical remission following single-agent or combination antibiotic therapy. Failure to respond to this treatment is likely to be due to the presence of resistant organisms. Faecal coliform sensitivity testing to identify an appropriate alternative antibiotic should be performed. About 5–19% of patients will develop chronic

relapsing pouchitis [Madiba and Bartolo, 2001; Mowschenson *et al.* 2000; Hurst *et al.* 1998]. These should be treated with maintenance therapy to maintain symptomatic remission. In those patients where maintenance therapy fails to control symptoms indefinite diversion with an ileostomy with or without pouch excision should be considered.

Conflict of interest statement

The Authors declare that there are no conflicts of interest.

Financial support

References

Andoh, A., Sakata, S., Koizumi, Y., Mitsuyama, K., Fujiyama, Y. and Benno, Y. (2007) Terminal restriction fragment length polymorphism analysis of the diversity of fecal microbiota in patients with ulcerative colitis. *Inflamm Bowel Dis* 13: 955–962.

Barton, J.G., Paden, M.A., Lane, M. and Postier, R.G. (2001) Comparison of postoperative outcomes in ulcerative colitis and familial polyposis patients after ileoanal pouch operations. *Am J Surg* 182: 616–620.

Bell, A.J., Nicholls, R.J., Forbes, A., Ellis, H.J. and Ciclitira, P.J. (2004) Human lymphocyte stimulation with pouchitis flora is greater than with flora from a healthy pouch but is suppressed by metronidazole. *Gut* 53: 1801–1805.

Bell, A.J., Price, A.B., Forbes, A., Ciclitira, P.J., Groves, C. and Nicholls, R.J. (2006) Pre-pouch ileitis: a disease of the ileum in ulcerative colitis after restorative proctocolectomy. *Colorectal Dis* 8: 402–410.

Belluzzi, A., Serrani, M., Roda, G., Bianchi, M.L., Castellani, L., Grazia, M. *et al.* (2010) Pilot study: the use of sulfasalazine for the treatment of acute pouchitis. *Aliment Pharmacol Ther* 31(2): 228–232.

Calabrese, C., Fabbri, A., Gionchetti, P., Rizzello, F., Morselli, C., Liguori, G. *et al.* (2007) Controlled study using wireless capsule endoscopy for the evaluation of the small intestine in chronic refractory pouchitis. *Aliment Pharmacol Ther* 25: 1311–1316.

Calabrese, C., Gionchetti, P., Rizzello, F., Liguori, G., Gabusi, V., Tambasco, R. *et al.* (2008) Short-term treatment with infliximab in chronic refractory pouchitis and ileitis. *Aliment Pharmacol Ther* 27: 759–764.

Carter, M.J., Lobo, A.J. and Travis, S.P. (2004) Guidelines for the management of inflammatory bowel disease in adults. *Gut* 53(Suppl 5): V1–V16.

Casadesus, D., Tani, T., Wakai, T., Maruyama, S., Iiai, T., Okamoto, H. *et al.* (2007) Possible role of human cytomegalovirus in pouchitis after proctocolectomy with ileal pouch-anal anastomosis in patients with ulcerative colitis. *World J Gastroenterol* 13: 1085–1089.

de Silva, H.J., Ireland, A., Kettlewell, M., Mortensen, N. and Jewell, D.P. (1989) Short-chain fatty acid irrigation in severe pouchitis. *N Engl J Med* 321: 1416–1417.

Dozois, R.R., Kelly, K.A., Welling, D.R., Gordon, H., Beart Jr, R.W., Wolff, B.G. *et al.* (1989) Ileal pouch-anal anastomosis: comparison of results in familial adenomatous polyposis and chronic ulcerative colitis. *Ann Surg* 210: 268–271.

Duffy, M., O'Mahony, L., Coffey, J.C., Collins, J.K., Shanahan, F., Redmond, H.P. *et al.* (2002) Sulfate-reducing bacteria colonize pouches formed for ulcerative colitis but not for familial adenomatous polyposis. *Dis Colon Rectum* 45: 384–388.

Elewaut, D., De Keyser, F., Cuvelier, C., Lazarovits, A.I., Mielants, H., Verbruggen, G. *et al.* (1998) Distinctive activated cellular subsets in colon from patients with Crohn's disease and ulcerative colitis. *Scand J Gastroenterol* 33: 743–748.

Falk, A., Olsson, C., Ahrne, S., Molin, G., Adawi, D. and Jeppsson, B. (2007) Ileal pelvic pouch microbiota from two former ulcerative colitis patients, analysed by DNA-based methods, were unstable over time and showed the presence of Clostridium perfringens. *Scand J Gastroenterol* 42: 973–985.

Fazio, V.W., Ziv, Y., Church, J.M., Oakley, J.R., Lavery, I.C., Milsom, J.W. *et al.* (1995) Ileal pouch-anal anastomoses complications and function in 1005 patients. *Ann Surg* 222: 120–127.

Ferrante, M., D'Haens, G., Dewit, O., Baert, F., Holvoet, J., Geboes, K. *et al.* (2010) Efficacy of infliximab in refractory pouchitis and Crohn's disease-related complications of the pouch: a Belgian case series. *Inflamm Bowel Dis* 16(2): 243–249.

Fiocchi, C. (1998) Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* 115: 182–205.

Frank, D.N., St Amand, A.L., Feldman, R.A., Boedeker, E.C., Harpaz, N. and Pace, N.R. (2007) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A* 104: 13780–13785.

Fukushima, K., Fujii, H., Yamamura, T., Sugita, A., Kameoka, S., Nagawa, H. *et al.* (2007) Pouchitis atlas for objective endoscopic diagnosis. *J Gastroenterol* 42: 799–806.

Gately, M.K., Renzetti, L.M., Magram, J., Stern, A.S., Adorini, L., Gubler, U. *et al.* (1998) The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu Rev Immunol* 16: 495–521.

Giacomelli, R., Passacantando, A., Parzanese, I., Vernia, P., Klidara, N., Cucinelli, F. *et al.* (1998) Serum levels of soluble CD30 are increased in

ulcerative colitis (UC) but not in Crohn's disease (CD). *Clin Exp Immunol* 111: 532–535.

Gionchetti, P., Rizzello, F., Morselli, C., Poggioli, G., Tambasco, R., Calabrese, C. *et al.* (2007a) High-dose probiotics for the treatment of active pouchitis. *Dis Colon Rectum* 50: 2075–2082.

Gionchetti, P., Rizzello, F., Poggioli, G., Morselli, C., Lammers, K.M. and Campieri, M. (2005) Probiotic therapy to prevent pouchitis onset. *Dis Colon Rectum* 48: 1493–1494.

Gionchetti, P., Rizzello, F., Poggioli, G., Pierangeli, F., Laureti, S., Morselli, C. *et al.* (2007b) Oral budesonide in the treatment of chronic refractory pouchitis. *Aliment Pharmacol Ther* 25: 1231–1236.

Gionchetti, P., Rizzello, F., Venturi, A., Brigidi, P., Matteuzzi, D., Bazzocchi, G. *et al.* (2000) Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology* 119: 305–309.

Gionchetti, P., Rizzello, F., Venturi, A., Ferretti, M., Brignola, C., Peruzzo, S. *et al.* (1997) Long-term efficacy of bismuth carbomer enemas in patients with treatment-resistant chronic pouchitis. *Aliment Pharmacol Ther* 11: 673–678.

Gophna, U., Sommerfeld, K., Gophna, S., Doolittle, W.F. and Veldhuyzen van Zanten, S.J. (2006) Differences between tissue-associated intestinal microfloras of patients with Crohn's disease and ulcerative colitis. *J Clin Microbiol* 44: 4136–4141.

Gosselink, M.P., Schouten, W.R., van Lieshout, L.M., Hop, W.C., Laman, J.D. and Ruseler-van Embden, J.G. (2004) Eradication of pathogenic bacteria and restoration of normal pouch flora: comparison of metronidazole and ciprofloxacin in the treatment of pouchitis. *Dis Colon Rectum* 47: 1519–1525.

Hahnloser, D., Pemberton, J.H., Wolff, B.G., Larson, D.R., Crownhart, B.S. and Dozois, R.R. (2007) Results at up to 20 years after ileal pouch-anal anastomosis for chronic ulcerative colitis. *Br J Surg* 94: 333–340.

Hayashi, H., Sakamoto, M. and Benno, Y. (2002) Phylogenetic analysis of the human gut microbiota using 16S rDNA clone libraries and strictly anaerobic culture-based methods. *Microbiol Immunol* 46: 535–548.

Hurst, R.D., Chung, T.P., Rubin, M. and Michelassi, F. (1998) The implications of acute pouchitis on the long-term functional results after restorative proctocolectomy. *Inflamm Bowel Dis* 4: 280–284.

Isaacs, K.L., Sandler, R.S., Abreu, M., Picco, M.F., Hanauer, S.B., Bickston, S.J. *et al.* (2007) Rifaximin for the treatment of active pouchitis: a randomized, double-blind, placebo-controlled pilot study. *Inflamm Bowel Dis* 13: 1250–1255.

Iwata, T., Yamamoto, T., Umegae, S. and Matsumoto, K. (2007) Pouchitis and pre-pouch ileitis developed after restorative proctocolectomy for ulcerative colitis: a case report. *World J Gastroenterol* 13: 643–646.

Iwaya, A., Iiai, T., Okamoto, H., Ajioka, Y., Yamamoto, T., Asahara, T. *et al.* (2006) Change in the bacterial flora of pouchitis. *Hepatogastroenterology* 53: 55–59.

Joelsson, M., Andersson, M., Bark, T., Gullberg, K., Hallgren, T., Jiborn, H. *et al.* (2001) Allopurinol as prophylaxis against pouchitis following ileal pouch-anal anastomosis for ulcerative colitis. A randomized placebo-controlled double-blind study. *Scand J Gastroenterol* 36: 1179–1184.

Johnson, E., Carlsen, E., Nazir, M. and Nygaard, K. (2001) Morbidity and functional outcome after restorative proctocolectomy for ulcerative colitis. *Eur J Surg* 167: 40–45.

Johnson, M.W., Rogers, G.B., Bruce, K.D., Lilley, A.K., von Herbay, A., Forbes, A. *et al.* (2009) Bacterial community diversity in cultures derived from healthy and inflamed ileal pouches after restorative proctocolectomy. *Inflamm Bowel Dis* 15(12): 1803–1811.

Kartheuser, A.H., Parc, R., Penna, C.P., Tiret, E., Frileux, P., Hannoun, L. *et al.* (1996) Ileal pouch-anal anastomosis as the first choice operation in patients with familial adenomatous polyposis: a ten-year experience. *Surgery* 119: 615–623.

Kmiot, W.A., Youngs, D., Tudor, R., Thompson, H. and Keighley, M.R. (1993) Mucosal morphology, cell proliferation and faecal bacteriology in acute pouchitis. *Br J Surg* 80: 1445–1449.

Kobayashi, M., Fitz, L., Ryan, M., Hewick, R.M., Clark, S.C., Chan, S., Loudon, R. *et al.* (1989) Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. *J Exp Med* 170: 827–845.

Komanduri, S., Gillevet, P.M., Sikaroodi, M., Mutlu, E. and Keshavarzian, A. (2007) Dysbiosis in pouchitis: evidence of unique microfloral patterns in pouch inflammation. *Clin Gastroenterol Hepatol* 5: 352–360.

Kuhbacher, T., Ott, S.J., Helwig, U., Mimura, T., Rizzello, F., Kleessen, B. *et al.* (2006) Bacterial and fungal microbiota in relation to probiotic therapy (VSL#3) in pouchitis. *Gut* 55: 833–841.

Kuisma, J., Jarvinen, H., Kahri, A. and Farkkila, M. (2004) Factors associated with disease activity of pouchitis after surgery for ulcerative colitis. *Scand J Gastroenterol* 39: 544–548.

Kuisma, J., Mentula, S., Luukkonen, P., Jarvinen, H., Kahri, A. and Farkkila, M. (2003) Factors associated with ileal mucosal morphology and inflammation in patients with ileal pouch-anal anastomosis for ulcerative colitis. *Dis Colon Rectum* 46: 1476–1483.

Lim, M., Adams, J.D., Wilcox, M., Finan, P., Sagar, P. and Burke, D. (2009) An assessment of bacterial dysbiosis in pouchitis using terminal restriction fragment length polymorphisms of 16S ribosomal DNA from pouch effluent microbiota. *Dis Colon Rectum* 52: 1492–1500.

Lim, M., Sagar, P., Finan, P., Burke, D. and Schuster, H. (2006) Dysbiosis and pouchitis. *Br J Surg* 93: 1325–1334.

Lohmuller, J.L., Pemberton, J.H., Dozois, R.R., Ilstrup, D. and van, H.J. (1990) Pouchitis and extra-intestinal manifestations of inflammatory bowel disease after ileal pouch-anal anastomosis. *Ann Surg* 211: 622–627.

Madden, M.V., McIntyre, A.S. and Nicholls, R.J. (1994) Double-blind crossover trial of metronidazole versus placebo in chronic unremitting pouchitis. *Dig Dis Sci* 39: 1193–1196.

Madiba, T.E. and Bartolo, D.C. (2001) Pouchitis following restorative proctocolectomy for ulcerative colitis: incidence and therapeutic outcome. *J R Coll Surg Edinb* 46: 334–337.

McLaughlin, S.D., Clark, S.K., Bell, A.J., Tekkis, P.P., Ciclitira, P.J. and Nicholls, R.J. (2008a) An open study of antibiotics for the treatment of pre-pouch ileitis following restorative proctocolectomy with ileal pouch-anal anastomosis. *Aliment Pharmacol Ther* 27(10): 895–909 [Epub 2008 Feb 9. Review].

McLaughlin, S.D., Clark, S.K., Shafi, S., Petrovksa, L., Tekkis, P.P., Ciclitira, P.J. *et al.* (2009a) Fecal coliform testing to identify effective antibiotic therapies for patients with antibiotic-resistant pouchitis. *Clin Gastroenterol Hepatol* 7: 545–548.

McLaughlin, S.D., Clark, S.K., Tekkis, P.P., Ciclitira, P.J. and Nicholls, R.J. (2008b) Review article: restorative proctocolectomy, indications, management of complications and follow-up—a guide for gastroenterologists. *Aliment Pharmacol Ther* 27: 895–909.

McLaughlin, S.D., Clark, S.K., Tekkis, P.P., Ciclitira, P.J. and Nicholls, R.J. (2009b) An open study of maintenance antibiotic therapy for chronic antibiotic dependent pouchitis; efficacy, complications and outcome. *Clin Gastroenterol Hepatol* 7(5): 545–548.

McLaughlin, S.D., Walker, A.W., Churcher, C., Clark, S.K., Tekkis, P.P., Johnson, M.W. *et al.* (2010) The bacteriology of pouchitis: a molecular phylogenetic analysis using 16srRNA gene cloning and sequencing. *Ann Surg*, in press.

Mimura, T., Rizzello, F., Helwig, U., Poggioli, G., Schreiber, S., Talbot, I.C. *et al.* (2004) Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut* 53: 108–114.

Moskowitz, R.L., Shepherd, N.A. and Nicholls, R.J. (1986) An assessment of inflammation in the reservoir after restorative proctocolectomy with ileoanal ileal reservoir. *Int J Colorectal Dis* 1: 167–174.

Mowschenson, P.M., Critchlow, J.F. and Peppercorn, M.A. (2000) Ileoanal pouch operation: long-term outcome with or without diverting ileostomy. *Arch Surg* 135: 463–465.

Nicholls, R.J., Belliveau, P., Neill, M., Wilks, M. and Tabaqchali, S. (1981) Restorative proctocolectomy with ileal reservoir: a pathophysiological assessment. *Gut* 22: 462–468.

Nyam, D.C., Brillant, P.T., Dozois, R.R., Kelly, K.A., Pemberton, J.H. and Wolff, B.G. (1997) Ileal pouch-anal canal anastomosis for familial adenomatous polyposis: early and late results. *Ann Surg* 226: 514–519.

O'Connell, P.R., Rankin, D.R., Weiland, L.H. and Kelly, K.A. (1986) Enteric bacteriology, absorption, morphology and emptying after ileal pouch-anal anastomosis. *Br J Surg* 73: 909–914.

Ohge, H., Furne, J.K., Springfield, J., Rothenberger, D.A., Madoff, R.D. and Levitt, M.D. (2005) Association between fecal hydrogen sulfide production and pouchitis. *Dis Colon Rectum* 48: 469–475.

Ott, S.J., Musfeldt, M., Wenderoth, D.F., Hampe, J., Brant, O., Folsch, U.R. *et al.* (2004) Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut* 53: 685–693.

Pardi, D.S. and Sandborn, W.J. (2006) Systematic review: the management of pouchitis. *Aliment Pharmacol Ther* 23: 1087–1096.

Pfau, P.R. and Lichtenstein, G.R. (2000) Cytomegalovirus infection as a cause of ileoanal pouchitis. *Dis Colon Rectum* 43: 113–114.

Romanos, J., Samarasekera, D.N., Stebbing, J.F., Jewell, D.P., Kettlewell, M.G. and Mortensen, N.J. (1997) Outcome of 200 restorative proctocolectomy operations: the John Radcliffe Hospital experience. *Br J Surg* 84: 814–818.

Rowley, S., Candinas, D., Mayer, A.D., Buckels, J.A., McMaster, P. and Keighley, M.R. (1995) Restorative proctocolectomy and pouch anal anastomosis for ulcerative colitis following orthotopic liver transplantation. *Gut* 37: 845–847.

Ruseler-van Embden, J.G., Schouten, W.R. and van Lieshout, L.M. (1994) Pouchitis: result of microbial imbalance? *Gut* 35: 658–664.

Sambuelli, A., Boerr, L., Negreira, S., Gil, A., Camartino, G., Huernos, S. *et al.* (2002) Budesonide enema in pouchitis—a double-blind, double-dummy, controlled trial. *Aliment Pharmacol Ther* 16: 27–34.

Sandborn, W.J. and Pardi, D.S. (2004) Clinical management of pouchitis. *Gastroenterology* 127: 1809–1814.

Sandborn, W.J., Tremaine, W.J., Batts, K.P., Pemberton, J.H. and Phillips, S.F. (1994) Pouchitis after ileal pouch-anal anastomosis: a Pouchitis Disease Activity Index. *Mayo Clin Proc* 69: 409–415.

Sandborn, W.J., Tremaine, W.J., Batts, K.P., Pemberton, J.H., Rossi, S.S., Hofmann, A.F. *et al.* (1995) Fecal bile acids, short-chain fatty acids, and bacteria after ileal pouch-anal anastomosis do not differ in patients with pouchitis. *Dig Dis Sci* 40: 1474–1483.

Santavirta, J., Mattila, J., Kokki, M. and Matikainen, M. (1991) Mucosal morphology and faecal bacteriology after ileoanal anastomosis. *Int J Colorectal Dis* 6: 38–41.

Sartor, R.B. (1991) Pathogenetic and clinical relevance of cytokines in inflammatory bowel disease. *Immunol Res* 10: 465–471.

Sartor, R.B. (2008) Microbial influences in inflammatory bowel diseases. *Gastroenterology* 134: 577–594.

Seksik, P., Rigottier-Gois, L., Gramet, G., Sutren, M., Pochart, P., Marteau, P. *et al.* (2003) Alterations of the dominant faecal bacterial groups in patients with Crohn's disease of the colon. *Gut* 52: 237–242.

Shen, B., Achkar, J.P., Connor, J.T., Ormsby, A.H., Remzi, F.H., Bevins, C.L. *et al.* (2003) Modified pouchitis disease activity index: a simplified approach to the diagnosis of pouchitis. *Dis Colon Rectum* 46: 748–753.

Shen, B., Achkar, J.P., Lashner, B.A., Ormsby, A.H., Remzi, F.H., Brzezinski, A. *et al.* (2001) A randomized clinical trial of ciprofloxacin and metronidazole to treat acute pouchitis. *Inflamm Bowel Dis* 7: 301–305.

Shen, B., Brzezinski, A., Fazio, V.W., Remzi, F.H., Achkar, J.P., Bennett, A.E. *et al.* (2005) Maintenance therapy with a probiotic in antibiotic-dependent pouchitis: experience in clinical practice. *Aliment Pharmacol Ther* 22: 721–728.

Shen, B., Fazio, V.W., Remzi, F.H., Bennett, A.E., Brzezinski, A., Lopez, R. *et al.* (2006) Risk factors for clinical phenotypes of Crohn's disease of the ileal pouch. *Am J Gastroenterol* 101: 2760–2768.

Shen, B., Fazio, V.W., Remzi, F.H., Bennett, A.E., Lopez, R., Brzezinski, A. *et al.* (2007a) Combined ciprofloxacin and tinidazole therapy in the treatment of chronic refractory pouchitis. *Dis Colon Rectum* 50(4): 498–508.

Shen, B., Fazio, V.W., Remzi, F.H., Bennett, A.E., Lopez, R., Lavery, I.C. *et al.* (2007b) Effect of withdrawal of nonsteroidal anti-inflammatory drug use on ileal pouch disorders. *Dig Dis Sci* 52(12): 3321–3328 [Epub 2007 Apr 5].

Shen, B., Remzi, F.H., Lopez, A.R. and Queener, E. (2008) Rifaximin for maintenance therapy in antibiotic-dependent pouchitis. *BMC Gastroenterol* 8: 26.

Slatter, C., Girgis, S., Huynh, H. and El Matary, W. (2008) Pre-pouch ileitis after colectomy in paediatric ulcerative colitis. *Acta Paediatr* 97: 381–383.

Sokol, H., Lepage, P., Seksik, P., Dore, J. and Marteau, P. (2006) Temperature gradient gel electrophoresis of fecal 16S rRNA reveals active Escherichia coli in the microbiota of patients with ulcerative colitis. *J Clin Microbiol* 44: 3172–3177.

Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermudez-Humaran, L.G., Gratadoux, J.J. *et al.* (2008) Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci U S A* 105: 16731–16736.

Sokol, H., Seksik, P., Furet, J.P., Firmesse, O., Nion-Larmurier, I., Beaugerie, L. *et al.* (2009) Low counts of Faecalibacterium prausnitzii in colitis microbiota. *Inflamm Bowel Dis* 15: 1183–1189.

Stahlberg, D., Gullberg, K., Liljeqvist, L., Hellers, G. and Lofberg, R. (1996) Pouchitis following pelvic pouch operation for ulcerative colitis. Incidence, cumulative risk, and risk factors. *Dis Colon Rectum* 39: 1012–1018.

Stallmach, A., Schafer, F., Hoffmann, S., Weber, S., Muller-Molaian, I., Schneider, T. *et al.* (1998) Increased state of activation of CD4 positive T cells and elevated interferon gamma production in pouchitis. *Gut* 43: 499–505.

Suau, A., Bonnet, R., Sutren, M., Godon, J.J., Gibson, G.R., Collins, M.D. *et al.* (1999) Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Appl Environ Microbiol* 65: 4799–4807.

Thomas, P.D., Forbes, A., Nicholls, R.J. and Ciclitira, P.J. (2001) Altered expression of the lymphocyte activation markers CD30 and CD27 in patients with pouchitis. *Scand J Gastroenterol* 36: 258–264.

Thomas, P.D., Forbes, A., Price, A.B., Nicholls, R.J. and Ciclitira, P.J. (2002) Differential expression of cell adhesion molecules within inflamed ileal pouch mucosa: relationship to recruited cell subtypes. *Eur J Gastroenterol Hepatol* 14: 137–144.

Tremaine, W.J., Sandborn, W.J., Wolff, B.G., Carpenter, H.A., Zinsmeister, A.R. *et al.* (1997) Bismuth carbomer foam enemas for active chronic pouchitis: a randomized, double-blind, placebo-controlled trial. *Aliment Pharmacol Ther* 11: 1041–1046.

Wolf, J.M., Achkar, J.P., Lashner, B.A., Delaney, C.P., Petras, R.E., Goldblum, J.R. *et al.* (2004) Afferent limb ulcers predict Crohn's disease in patients with ileal pouch-anal anastomosis. *Gastroenterology* 126: 1686–1691.

Wolf, S.F., Temple, P.A., Kobayashi, M., Young, D., Dicig, M., Lowe, L. *et al.* (1991) Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. *J Immunol* 146: 3074–3081.

Zins, B.J., Sandborn, W.J., Penna, C.R., Landers, C.J., Targan, S.R., Tremaine, W.J. *et al.* (1995) Pouchitis disease course after orthotopic liver transplantation in patients with primary sclerosing cholangitis and an ileal pouch-anal anastomosis. *Am J Gastroenterol* 90: 2177–2181.

Zoetendal, E.G., Rajilic-Stojanovic, M. and De Vos, W.M. (2008) High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. *Gut* 57: 1605–1615.