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## Faecal Microbiota Transplantation for Treatment of Chronic Pouchitis

*Role of Gut Microbiota in Disease Mechanism and Treatment of Pouchitis*

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**FAECAL MICROBIOTA  
TRANSPLANTATION FOR TREATMENT  
OF CHRONIC POUCHITIS**

ROLE OF GUT MICROBIOTA IN DISEASE MECHANISM  
AND TREATMENT OF POUCHITIS

**BY  
SABRINA JUST KOUSGAARD**

DISSERTATION SUBMITTED 2023



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by

Sabrina Just Kousgaard



**AALBORG UNIVERSITY**  
DENMARK

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Sabrina Just Kousgaard, Aalborg 2023

# ENGLISH SUMMARY

Pouchitis is an inflammation in the ileal pouch-anal anastomosis (IPAA) after restorative proctocolectomy often seen after the surgical treatment of ulcerative colitis (UC). Pouchitis is the most frequent long-term complication after IPAA with an incidence of 14%-59% in Western countries. Antibiotic treatment with ciprofloxacin and/or metronidazole is the first line of treatment, which often fails in chronic pouchitis. Therapeutic options for chronic pouchitis are few, which may in the end lead to pouch failure and surgical removal of the ileal pouch.

The pathogenesis of pouchitis is only poorly understood, but pouchitis is hypothesised to be caused by immune dysregulation, genetic predispositions and imbalance in the gut microbiota. Changes in the intestinal microbial environment have been associated with pouchitis, and studies have found an altered gut microbiota in patients with pouchitis compared with healthy persons. Faecal microbiota transplantation (FMT) can restore an imbalanced gut microbiota and is now a well-established treatment for recurrent *Clostridioides difficile* infection. Several clinical trials have found solid evidence for the use of FMT to induce clinical remission in mild to moderate UC. Theoretically, FMT could be a new therapeutic strategy for patients with chronic pouchitis. The aim of the studies included in this thesis was to investigate the influence of the gut microbiota on chronic pouchitis and the use of FMT as a new treatment strategy for patients with chronic pouchitis.

We investigated the gut microbiota profile in patients with chronic pouchitis compared with patients with a non-inflamed IPAA, patients with familial adenomatous polyposis and with healthy individuals. We found that the gut microbiota profile in patients with chronic pouchitis differed from that of patients with a normally functioning IPAA and that of healthy individuals. Whereas bacteria of the genus *Bacteroides* were mainly associated with healthy individuals, bacteria of the family *Enterobacteriaceae* and particularly genus *Escherichia* were primarily associated with patients with chronic pouchitis. Furthermore, patients with chronic pouchitis had lower microbial diversity and richness than patients with a normally functioning IPAA.

To evaluate published studies testing FMT in the treatment of chronic pouchitis, we conducted a systematic literature review. In the review, a total of 65 patients were treated with FMT in the included studies. Pooled estimates of clinical response and remission after FMT were 32% and 23%, respectively. The only included randomised controlled trial (RCT) found no benefit of FMT compared with placebo. The studies were very heterogeneous concerning donor selection, stool processing, delivery, treatment length, scoring of treatment efficacy and follow-up. A few studies found increased microbial diversity and higher resemblance to the donor microbiota in patients after FMT.

In addition, we tested the use of non-pooled multi-donor FMT in the treatment of patients with chronic pouchitis. We found a clinical remission rate of 44% and 40% in the open-label pilot study and RCT, respectively. However, compared with patients treated with placebo in the RCT, the two groups showed no difference in clinical remission rate with a relative risk of 1 (95% CI 0.55-1.81). The gut microbiota in faecal samples from patients treated with FMT and their donors were analysed. After FMT treatment in the open-label pilot study, richness and marginal diversity increased in patients' samples ( $p=0.004$  and  $p=0.16$ ), and a higher similarity to the faecal donors' microbiota was found ( $p=0.004$ ). In the RCT, the microbial community of donor samples constitutes more genera and less domination by a single genus with high abundance of genus *Faecalibacterium*, the unclassified genus from the family *Lachnospiraceae*, and genus *Ruminococcus*. In contrast, the faecal patient microbiome is less diverse, and some patients' faecal microbiome is almost entirely the genus *Escherichia* or *Streptococcus*. After placebo treatment, the microbial composition is generally similar with few overall compositional changes, but the microbial composition after FMT treatment did alter the microbiome slightly and increased the median similarity to the faecal donor microbiome.

In conclusion, the composition of the gut microbiota in patients with chronic pouchitis differs from that of patients with a normally functioning IPAA and that of healthy individuals. Some patients with chronic pouchitis improved clinically after FMT, but no clinical benefit of FMT from a healthy faecal donor compared with placebo was observed. Further research into the mechanism of the gut microbiota and FMT in patients with chronic pouchitis is needed, including selection of faecal donors.

# DANSK RESUME

Pouchitis er en inflammatorisk tilstand i den ileo-pouch-anal-anastomose (IPAA), som er lavet efter proktokolektomi i forbindelse med kirurgisk behandling af colitis ulcerosa (UC). I den vestlige verden er pouchitis den mest almindelige langtidskomplikation, og incidensen heraf er 14%-59%. Den primære behandling af pouchitis er antibiotisk behandling med ciprofloxacin og/eller metronidazol, som i tilfælde af kronisk pouchitis ofte er mangelfuld. Mulighederne for behandling af kronisk pouchitis er begrænsede, hvilket i sidste ende kan føre til fjernelse af pouchen.

Årsagen til udvikling af pouchitis kendes ikke, men pouchitis formodes at skyldes et sammenspil mellem flere faktorer, herunder et dysreguleret immunrespons, genetisk prædisposition og ubalance i tarmmikrobiota. Ændringer i det mikrobielle tarmmiljø er blevet associeret med pouchitis, og studier har fundet et ændret tarmmikrobiota hos patienter med pouchitis sammenlignet med raske. Fæcestransplantation (FMT) kan genoprette ubalancen i tarmens mikrobiota og er nu en etableret behandling til patienter med tilbagevendende infektion med *Clostridioides difficile*. Flere kliniske forsøg har fundet substantiel evidens for brugen af FMT til patienter med mild til moderat UC. FMT kunne teoretisk være en ny behandlingsmulighed hos patienter med kronisk pouchitis. Formålet med studierne i denne afhandling er at undersøge betydningen af tarmmikrobiota ved kronisk pouchitis, samt at vise, om FMT kunne være en ny behandlingsmulighed hos patienter med kronisk pouchitis.

Vi undersøgte tarmmikrobiota-profilen hos patienter med kronisk pouchitis sammenlignet med profilen hos patienter uden en inflammatorisk IPAA, familiær adenomatøs polypose og raske individer. Tarmmikrobiomet hos patienter med kronisk pouchitis adskilte sig fra tarmmikrobiomet hos patienter uden en inflammatorisk IPAA og hos raske individer. Særligt genus *Bacteroides* var associeret med raske individer, imens familie *Enterobacteriaceae* og genus *Escherichia* var associeret med kronisk pouchitis. Desuden havde patienter med kronisk pouchitis lavere mikrobiel diversitet og rigdom end patienter uden en inflammatorisk IPAA.

Vi udførte en systematisk litteraturgennemgang for at evaluere de publicerede studier, der har undersøgt FMT til behandling af kronisk pouchitis. Man havde i studierne inkluderet i alt 65 patienter, der blev behandlet med FMT. Det samlede estimat viste, at klinisk respons blev opnået hos 32% af patienterne, og 23% af patienterne opnåede klinisk remission efter FMT. Kun et randomiseret forsøg (RCT) blev inkluderet, og de fandt ingen fordel ved FMT sammenlignet med placebo. Studierne var meget heterogene, både hvad angik donorudvælgelse, afføringsprocessering, behandlingstidslængde, administrationsvej, scoring af behandlingseffekt og opfølgning. Få studier fandt øget mikrobiel diversitet og større lighed med donors tarmmikrobiota i patienternes prøver efter FMT.

Vi undersøgte brugen af multidonor-FMT til patienter med kronisk pouchitis. Her fandt man en klinisk remissionsrate på henholdsvis 44% og 40% i open-label pilotstudiet og RCT-studiet. Da vi sammenlignede raten med patienter behandlet med placebo i RCT-studiet, fandt vi ingen forskel imellem de to grupper (relativ risiko 1 [95%CI 0.55-1.81]). Analyse af tarmmikrobiomet i pilotstudiet viste, at mikrobiel rigdom og marginal diversitet steg i patientprøverne efter FMT ( $p=0.004$  and  $p=0.16$ ), og der var en højere lighed med afføringsdonorernes mikrobiota efter FMT ( $p=0.004$ ). Sammensætningen af tarmmikrobiomet i RCT-studiet hos donorerne bestod mere af genera og var mindre domineret af enkle genus. Vi fandt høj tilstedeværelse af *Faecalibacterium*, den uklassificerede genus fra familien *Lachnospiraceae* og *Ruminococcus*. I modsætning hertil var diversiteten i tarmmikrobiomet hos patienterne mindre, da nogle prøver næsten kun bestod af genus *Escherichia* eller *Streptococcus*. Der var kun få overordnede ændringer i tarmmikrobiomet efter behandling med placebo, hvorimod sammensætningen af tarmmikrobiomet ændrede sig efter FMT og fik en større lighed med donorerens tarmmikrobiom.

Vi konkluderer, at tarmmikrobiota hos patienter med kronisk pouchitis er forskellig fra tarmmikrobiota fra patienter med en normalt fungerende IPAA og fra raske individer. Nogle patienter med kronisk pouchitis opnåede klinisk forbedring efter FMT, men der er ingen klinisk fordel ved FMT sammenlignet med placebo, når en rask afføringsdonor bruges.

Der er brug for større viden om mekanismen af tarmmikrobiomet og FMT hos patienter med kronisk pouchitis og om selektion af afføringsdonorer til denne patientgruppe.

# LIST OF STUDIES

The thesis is based on the following four studies:

- Paper I            **The Microbiota Profile in Inflamed and Non-Inflamed Ileal Pouch–Anal Anastomosis**
- Kousgaard SJ, Michaelsen TY, Nielsen HL, Kirk KF, Albertsen M, Thorlacius-Ussing O. *Microorganisms*. 2020 Oct 20;8(10):1611. doi: 10.3390/microorganisms8101611.
- Paper II            **Fecal Microbiota Transplantation in the Treatment of Chronic Pouchitis: A Systematic Review**
- Cold F\*, Kousgaard SJ\*, Halkjaer SI, Petersen AM, Nielsen HL, Thorlacius-Ussing O, Hansen LH. *Microorganisms*. 2020 Sep 18;8(9):1433. doi: 10.3390/microorganisms8091433.
- Paper III           **Clinical results and microbiota changes after faecal microbiota transplantation for chronic pouchitis: a pilot study**
- Kousgaard SJ, Michaelsen TY, Nielsen HL, Kirk KF, Brandt J, Albertsen M, Thorlacius-Ussing O. *Scand J Gastroenterol*. 2020 Apr;55(4):421-429. doi: 10.1080/00365521.2020.1748221.
- Paper IV            **Non-pooled Multi-donor Faecal Microbiota Transplantation to induce clinical remission in Patients with Chronic Pouchitis: A Randomised Placebo-Controlled Trial (MicroPouch)**
- Kousgaard SJ, Cold F, Halkjær SI, Petersen AM, Kjeldsen J, Hansen JM, Dall SM, Albertsen M, Nielsen HL, Kirk KF, Sønderkær M, Thorlacius-Ussing O. *In draft*.





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# CHAPTER 1. INTRODUCTION

## 1.1. ULCERATIVE COLITIS AND ILEAL POUCH ANAL ANASTOMOSIS

Ulcerative colitis (UC) is a chronic, relapsing disease with diffuse, nonspecific inflammation and ulcers in the gastrointestinal tract.<sup>1</sup> UC is classified as a chronic inflammatory bowel disease (IBD) together with Crohn's disease (CD). UC only affects the colon and rectum unlike CD which can affect the entire gastrointestinal tract. UC is characterised by continuous mucosal inflammation starting in the rectum and extending proximally.<sup>1</sup> UC can also affect the terminal ileum (termed backwash ileitis). The inflammation causes symptoms such as bleeding per rectum, increased frequency of defaecation, abdominal pain, urgency and tenesmus.<sup>2</sup>

UC is described to have a bimodal pattern of incidence. The primary onset peak is between the age of 15 and 30 years, and a smaller secondary peak of incidence is between the age of 50 and 70 years.<sup>3</sup> The incidence is in general increasing in Western and industrialised countries, with an incidence of approximately 14/100,000 people per year in Denmark, resulting in an estimated 35,000 individuals living with UC in Denmark.<sup>4</sup>

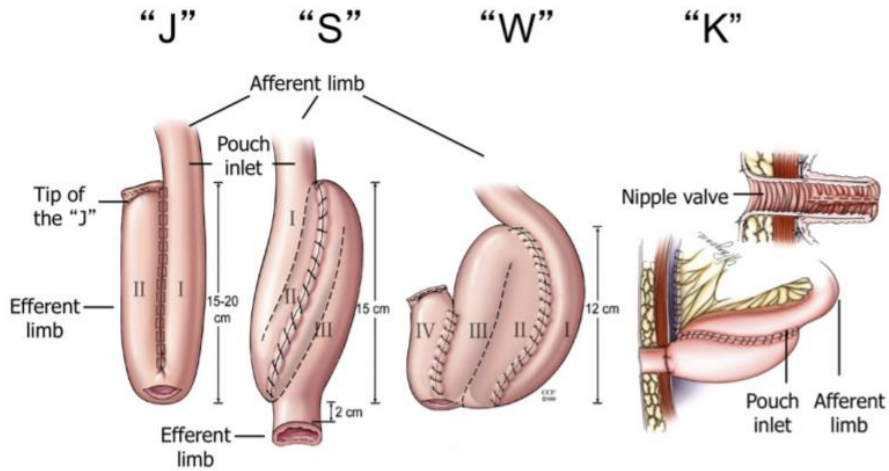
The aetiology of UC remains uncertain, but evidence indicates both innate and adaptive cellular immunity as key factors in disease pathogenesis together with an interaction of environmental, genetic and microbial factors.<sup>5,6</sup>

Medical treatment is the primary intervention in the treatment of UC with use of 5-aminosalicylic acid, corticosteroids, immunosuppressants and biologicals.<sup>7,8</sup> Most patients will respond to medical treatment; however, 20-30% of patients will require surgery with colectomy within their lifetime after medicinal therapeutic failure.<sup>9,10</sup>

### 1.1.1.1 Ileal pouch anal anastomosis

Total restorative proctocolectomy with ileal pouch anal anastomosis (IPAA) is the preferred surgical treatment of UC refractory to medical treatment.<sup>11</sup>

Different surgical techniques for an IPAA have been suggested. Commonly, a J-pouch is selected in which, initially, the entire colon and rectum is removed, preserving the sphincter and anus. This is followed by construction of a pouch formed like the letter J, by attaching the end of the terminal ileum to the anus.<sup>12,13</sup> The operation can be performed as either a 1st, 2nd or 3rd stage operation with or without construction of a temporary ileostomy.<sup>12,13</sup> The initial colectomy can be performed acutely in case of acute severe UC with risk of developing toxic megacolon.



**Figure 1.** Anatomy of different ileal pouches. Reprinted by permission from Elsevier Ltd., from *Shen B, Clinical Gastroenterology and Hepatology 2013 Dec;11:1538 – 1549. doi: 10.1016/j.cgh.2013.03.033.*

### 1.1.2. POUCHITIS

Pouchitis is characterised as inflammation in the IPAA formed after proctocolectomy.<sup>14</sup> Pouchitis is the most common complication after IPAA surgery. The incidence of pouchitis in Western countries is reported between 30% and 43% within the first year after IPAA surgery<sup>15–17</sup> and increases over time from surgery.<sup>18</sup> After IPAA surgery, up to 80% of patient will experience symptoms of pouchitis at some point in the disease course.<sup>19</sup> The rates of chronic pouchitis are approximately 15%–20% within the first five years after pouch surgery.<sup>18,20,21</sup> In contrast to the prevalence of pouchitis in patients with UC IPAA, the prevalence of pouchitis is only 6% in patients with a IPAA due to familial adenomatous polyposis (FAP).<sup>17,22</sup>

#### 1.1.2.1 Clinical findings

Clinical findings in pouchitis include a variety of symptoms, such as increased stool frequency and fluidity, abdominal cramping, rectal bleeding, urgency, tenesmus and night-time faecal incontinence.<sup>23</sup> Fever and extraintestinal manifestations can also occur.<sup>24,25</sup>

Pouchitis can be classified based on duration of symptoms, clinical course and response to medical treatment with antibiotics (Table 1).<sup>15</sup> Pouchitis is normally divided into acute ( $\leq 4$  weeks) and chronic ( $>4$  weeks) pouchitis, depending on the



duration of symptoms.<sup>15</sup> Furthermore, chronic antibiotic-refractory pouchitis is defined as chronic pouchitis non responsive to standard two-week antibiotic treatment.<sup>13</sup>

Subtypes of pouchitis		
Duration of symptoms	Acute	≤ 4 weeks
	Chronic	> 4 weeks
Symptom pattern	Infrequent	< 3 episodes per year
	Relapsing	≥ 3 episodes per year, or recurrence within one month of successful antibiotic therapy
Response to antibiotics	Antibiotic responsive	Responds to course of antibiotics
	Antibiotic dependent	Requires ongoing antibiotic therapy to maintain response
	Antibiotic refractory	Does not respond to standard course of antibiotics

**Table 1.** Classification categories for subtypes of pouchitis.

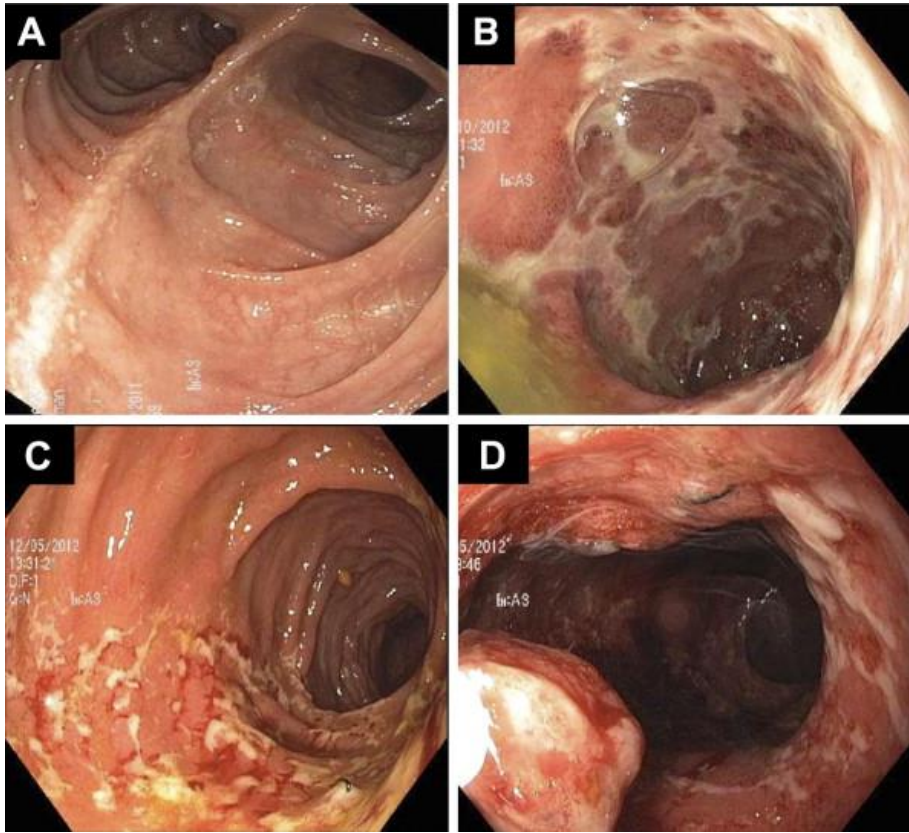
Other inflammatory diseases can also occur in the pouch. These include cuffitis, CD of the pouch and irritable pouch syndrome.<sup>26-28</sup> Patients can also initially be misclassified and later, after IPAA surgery, get a CD diagnosis.<sup>29</sup>

### 1.1.2.2 Endoscopic and histological findings

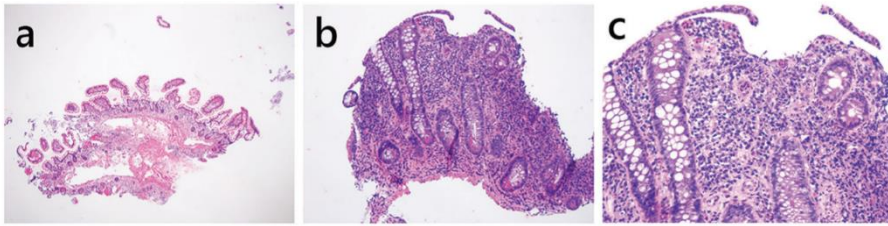
Pouchoscopy of patients with pouchitis can show non-specific endoscopic findings including diffuse erythema, oedema, granularity, friability, spontaneous or contact bleeding, loss of vascular pattern, mucous exudates, haemorrhage, erosions and ulcerations.<sup>30</sup>

Histological findings in biopsies from patients with pouchitis can be acute inflammation with polymorphonuclear leukocyte infiltration, crypt abscesses and ulceration, together with a chronic inflammatory infiltration.<sup>23</sup>

In some patients with pouchitis, a discrepancy may be seen between endoscopic and histological findings.<sup>31</sup>



**Figure 2.** Pouchoscopy findings. (A) Normal pouch inlet and tip of the J without inflammation. (B) Chronic pouchitis with ulceration. (C) Pouchitis with an ischaemic pattern, inflammation at the afferent limb side of the J pouch and normal mucosa at the efferent limb side. (D) Inflammatory polyp due to chronic mucosal inflammation in the pouch. Reprinted by permission from Elsevier Ltd., from *Shen B, Clinical Gastroenterology and Hepatology 2013 Dec;11:1538 –1549. doi: 10.1016/j.cgh.2013.03.033.*



**Figure 3.** Histological findings in pouch biopsies. (A) Histology of normal pouch. Small bowel mucosa with preserved and slender villous. Few mononuclear inflammatory cells are seen in the lamina propria. No sign of basal lymphoplasmacytosis, neutrophilic inflammation, epithelial injury, erosion or ulceration (H&E stain,  $\times 20$ ). (B, C) Chronic pouchitis of the small bowel mucosa with villous blunting, erosion, chronic and active inflammation (B: H&E,  $\times 100$ ; C: H&E stain,  $\times 200$ ). Reprinted by permission from Elmer Press, from *Gonzalo DH, Gastroenterology Res. 2016 Jun;9:29–38. doi: 10.14740/gr706e.*

### 1.1.2.3 Risk factors for pouchitis

Several risk factors have been associated with the development of pouchitis. In general, the disease-associated risk factors for development of pouchitis can be divided into pre-surgical, surgical and post-surgical risk factors.<sup>32</sup>

Pre-surgical risk factors	Surgical risk factors	Post-surgical risk factors
Pre-operative steroid use	Hand-sewn anastomosis	Non-steroidal anti-inflammatory drug use
Primary sclerosing cholangitis	Anastomosis placed <0.5 cm from dentate line	Iron-deficiency anaemia
Pulmonary comorbidity	S-pouch construction	Thrombocytosis
Extraintestinal manifestations		Long duration of time since pouch surgery
Backwash ileitis		Ulcerative gastroduodenal lesions
Pancolitis/extensive disease		
Steroid dependency		
First-degree relative with inflammatory bowel disease		
Chronic active inflammation of the appendix		
Presence of a concomitant autoimmune disorder		

**Table 2.** Risk factors for pouchitis.

### 1.1.3. DIAGNOSIS OF POUCHITIS

The diagnosis of pouchitis is based on symptom assessment, endoscopic findings and evaluation of biopsies from the pouch body and the afferent limb. During pouchoscopy, evaluation should encompass the afferent limb, inlet, tip of the J, proximal and distal pouch, anastomosis, rectal cuff, anal canal and perianal area.<sup>23</sup>

The 18-point Pouchitis Disease Activity Index (PDAI) is the most commonly used diagnostic tool to diagnose inflammation of the pouch.<sup>33</sup> The index includes sub-scores of clinical symptoms (cPDAI) (0–6 points), endoscopy (ePDAI) (0–6 points), and histology findings (hPDAI) (0–6 points). A total PDAI score of  $\geq 7$  points is considered diagnostic for pouchitis.<sup>23</sup> An alternative to the PDAI score is the modified PDAI (mPDAI) score, which only includes the clinical and endoscopy items of the PDAI score, with a score of  $\geq 5$  points considered diagnostic for pouchitis.<sup>34</sup>

PDAI score	
<b>Clinical</b>	
Stool frequency	
Usual stool frequency	0
1-2 stools/day > normal	1
3 or more stools/day > normal	2
Faecal urgency or abdominal cramps	
None	0
Occasional	1
Usual	2
Rectal bleeding	
None or rare	0
Daily	1
Fever	
Absent	0
Present	1
<b>Endoscopy inflammation</b>	
Oedema	1
Granularity	1
Friability	1
Loss of vascular pattern	1
Mucous exudates	1
Ulceration	1
<b>Histology</b>	
<b>Acute histologic inflammation</b>	
Polymorphonuclear leukocyte infiltration	
None	0
Mild	1
Moderate and crypt abscess	2
Severe and crypt abscess	3
Ulceration per low field (mean)	
None	0
<25%	1
25-50%	2
>50%	3

**Table 3.** Pouchitis Disease Activity Index score.

### 1.1.4. PATHOGENESIS OF POUCHITIS

Pouchitis is a heterogeneous disease. The clinical, endoscopic and histological appearance varies between patients, most likely because difference in host susceptibility and exposure to factors affecting the disease course.<sup>35</sup> The main proposed pathogenic mechanism of pouchitis is an interplay between recurrence of UC, immune dysregulation, genetic predispositions and microbiota dysbiosis.<sup>35</sup>

#### 1.1.4.1 Recurrence of UC in the IPAA

After pouch surgery, the terminal ileum used to form the pouch will change. Mucosal thickness is reduced, villi are lost or blunted and crypts develop to form a colon-like morphology, termed colonic metaplasia.<sup>35</sup> Histologically, presence of chronic inflammatory cell infiltrates (e.g. lymphocytes, plasma cells, eosinophils and histiocytes) are found in the lamina propria.<sup>36,37</sup>

The inflammation in pouchitis is interesting because both patients with UC and patients with FAP undergo the same surgical procedure, however only patients with UC undergoing IPAA surgery commonly develop pouchitis. This hypothesise that the underlying inflammatory response in patients with UC may be a factor in the pathogenesis of pouchitis.<sup>35</sup> It is suggested that changes of the ileal mucosa after construction of the pouch may lead to a recurrence of UC in the pouch in the form of pouchitis.<sup>35</sup>

Studies have found that patients with pouchitis have a higher degree of colonic metaplasia than patients without pouchitis.<sup>38,39</sup> Moreover, altered immune response impairs the epithelial layer, compromising the mucosal barrier, causing bacterial invasion into the mucosa due to the increased permeability of the intestinal epithelium.<sup>40,41</sup> Therefor pouchitis might be a recurrence of UC with development of a colonic-like morphology and a compromised mucosal barrier.<sup>35</sup>

Pathogen recognition receptors, expressed by the innate immune cells, identify and present luminal antigens to the immune system to sustain homeostasis in the intestinal ileum.<sup>35</sup> Increased expression of pathogen recognition receptors, such as nucleotide-binding oligomerization domain 2 (NOD2), toll-like receptor 2 and 4 (TLR2/TLR4) and transmembrane coreceptor CD14, have been found in the terminal ileum of patients with UC and in patients with a pouch.<sup>42-45</sup> This suggests that the terminal ileum, and the IPAA, has impaired immune tolerance in patients with UC, which might increase the risk for developing pouchitis.<sup>35</sup>

### 1.1.4.2 Immune dysregulation

The immune dysregulation in pouchitis is characterised by acute inflammation with polymorphonuclear infiltration in addition to the chronic inflammation with possible presence of ulceration and crypt abscesses.<sup>36,37</sup>

The ileal mucosal immune system is activated in patients after pouch construction and ileostomy takedown, and is markedly increased in pouch mucosa from patients with UC compared with patients with FAP.<sup>35</sup> The activated immune response in the non-inflamed pouch includes aberrant expression of TLRs, increased expression of Paneth-cell-specific defensin-5 mRNA, increased mucosal levels of interferon gamma (INF- $\gamma$ ), and increased transcription factor signal transducer and activator of transcription 1 (STAT-1).<sup>18,46</sup>

In case of pouchitis, activation of a non-specific inflammatory cascade is initiated with increased pro-inflammatory cytokines, including tumour necrosis factor alpha (TNF- $\alpha$ ), INF- $\gamma$ , interleukin 1 $\beta$ , 6 and 8 (IL-1 $\beta$ /IL-6/IL-8). Furthermore, levels of anti-inflammatory cytokine IL-10 are reduced.<sup>47-49</sup> Inflammatory cells like CD4<sup>+</sup> and CD40<sup>+</sup> T helper (Th) cells are present within the inflamed pouch mucosa in active pouchitis.<sup>50,51</sup> Additionally, CD4<sup>+</sup>/CD25<sup>+</sup> regulatory T cells (Tregs) and CD8<sup>+</sup>/HLA-DR<sup>+</sup> cytotoxic T cells are found to be increased in pouchitis.<sup>50</sup> Finally, CD19<sup>+</sup> and CD138<sup>+</sup> plasma cells/B-cells are increased in lamina propria of pouch mucosa with inflammation.<sup>52</sup>

Chronic pouchitis is characterised by upregulated innate and adaptive mucosal immune responses.<sup>53</sup> The upregulation of the immune response is characterised by increased expression of TLRs (TLR2 and TLR4), increased inflammatory cytokines from Th17 cells and reduced Tregs cells.<sup>15,49</sup>

Peyer's patches with gut-associated lymphoid tissue (GALT) and microfold (M) cells are found in the ileum. Antigens and luminal bacteria are transported by M cells to the immune cells in the mucosa, which can either stimulate or inhibit an immune response depending on presentation of pathogens or commensal organisms. Peyer's patches have an increased epithelial permeability, which makes Peyer's patches vulnerable to bacterial invasion.<sup>54</sup>

### 1.1.4.3 Genetic predisposition

Genetic background including ethnicity could be a factor contributing to the pathogenesis of pouchitis;<sup>35</sup> however, the few studies investigating the genetics in pouchitis are very heterogenous and potential associations need to be interpreted with caution.

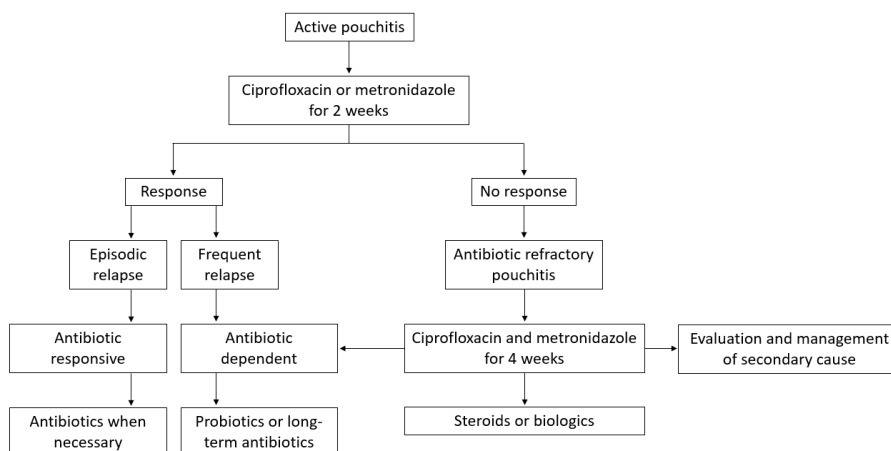
Caucasian ethnicity has been associated with pouchitis, including the Ashkenazi Jewish ethnicity and South Asian Caucasians living in the United Kingdom.<sup>55</sup> On the other hand, a study of African Americans and Caucasians described no difference in the incidence of chronic pouchitis between the two groups.<sup>56</sup>

The genetic predisposition to pouchitis is uncertain, but genetic polymorphisms could potentially be associated with pouchitis.<sup>35</sup> Well-designed studies on the genetics of pouchitis including heritability are scarce. The most compelling evidence is a possible association of an NOD2 variant (rs2066847) with pouchitis in the Caucasian population.<sup>57,58</sup> Furthermore, a significant association of the NOD2insC variant with chronic pouchitis was found (odds ratio (OR) 3.21, 95% confidence interval (CI) 1.38-7.47).<sup>57</sup>

Microbiota dysbiosis in pouchitis will be described later in the microbiota section.

### 1.1.5. TREATMENT OF POUCHITIS

Treatment of pouchitis is mainly empirical with only few placebo-controlled trials that may serve as guidance.<sup>59,60</sup> Treatment depends on the disease course and treatment effect for the individual patient, which is supported in the consensus statement from the International Ileal Pouch Consortium.<sup>61</sup>



**Figure 4.** Algorithm for treatment of pouchitis.

### 1.1.5.1 Treatment of acute pouchitis

Antibiotics have become the cornerstone in the treatment of pouchitis because it is believed that faecal stasis and bacterial overgrowth are important factors in acute pouchitis. This has led treatment with antibiotics as the preferred choice to treat patients, notably with ciprofloxacin or metronidazole as first-line treatment, often as monotherapy.<sup>15,23,24,62</sup>

### 1.1.5.2 Treatment of chronic pouchitis

Treatment of chronic pouchitis in general and chronic antibiotic-refractory pouchitis in particular is challenging and largely empirical. Treatment is based on combined antibiotic therapy, oral steroids or biologics.<sup>62</sup> First-line treatment of chronic pouchitis is a combination of two antibiotics, often ciprofloxacin with metronidazole for 4 weeks.<sup>23</sup> Long-term, low-dose antibiotics can be suggested for patients with antibiotic-dependent pouchitis not responding to probiotic maintenance therapy, which, however, can be associated with development of antibiotics resistance.<sup>63</sup>

A panel of IBD specialists investigated the overall appropriateness of different medical and surgical treatments including antibiotics, ileal release budesonide, probiotics, biologics and permanent ileostomy for chronic pouchitis using the RAND/UCLA appropriateness methodology.<sup>64</sup> In asymptomatic antibiotic-dependent chronic pouchitis, continuing antibiotic treatment and probiotics were rated as appropriate.<sup>64</sup> Inversely, in asymptomatic antibiotic-refractory chronic pouchitis, only no therapy was rated as appropriate but with discrepancy among the panel.<sup>64</sup> In both symptomatic antibiotic-dependent chronic pouchitis and antibiotic-refractory chronic pouchitis, ileal release budesonide and biological therapy (anti-TNF agents, vedolizumab and ustekinumab) were rated as appropriate.<sup>64</sup> Permanent ileostomy was found appropriate in symptomatic patients with chronic pouchitis failing treatment with both antibiotics, ileal-release budesonide, probiotics and biologics.<sup>64</sup>

### 1.1.6. PROGNOSIS OF POUCHITIS

The disease course of pouchitis varies among patients. One third of patients only experience a single episode of acute pouchitis, two thirds will experience several episodes of recurrent pouchitis, which for approximately one third will progress to chronic antibiotic-refractory pouchitis.<sup>65</sup>

Finally, patients with early-onset pouchitis and severe disease activity are at greater risk of developing chronic antibiotic-dependent pouchitis,<sup>66</sup> which can ultimately lead to pouch failure with a need for removal of the pouch and construction of a permanent



ileostomy. At 5 years after pouch surgery, the prevalence of pouch failure is 5% increasing to 9% at more than 10 years after surgery.<sup>67</sup>

## 1.2. MICROBIOTA

The human microbiota is an ecological community counting more than 100 trillion microorganisms existing in and on the human body.<sup>68</sup> The community includes bacteria, fungi, archaea and virus, which can function in a commensal, symbiotic or pathogenic relationship. The human microbiota has an important role in both health and disease. When looking into the genetic makeup of this community, the microbiome refers to the genomes of the microorganisms.<sup>69</sup>

### 1.2.1. THE GUT MICROBIOTA

The human microbiota is primarily located in the gut, termed gut microbiota. It is considered an essential organ, and the gut microbiome carries approximately 150 times more genes than the entire human genome.<sup>70</sup>

About 1,100 prevalent microbial species have been identified in the human gut microbiota, and it is estimated that at least 160 species are present in the gut microbiota of a single individual.<sup>71</sup> The composition of microorganisms in the gut microbiota varies with sex, ethnicity and age.<sup>72</sup> Dietary habits are also related to variations in the composition of the gut microbiota.<sup>73</sup>

In general, 99% of healthy individuals' gut microbiota is composed of *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria*, with *Firmicutes* and *Bacteroidetes*, which accounts for about 90% of the total composition.<sup>74</sup> Short-chain fatty acids (SCFAs), a gut microbiota-derived metabolite, are produced by *Firmicutes*, *Bacteroidetes* and oligosaccharide-fermenting bacteria as *Bifidobacterium* during fermentation of dietary plant fibres.<sup>75,76</sup> Gut microbiota-derived metabolite groups as SCFAs, bile acid metabolites and tryptophan metabolites have an important function in normal immune response and gut homeostasis.<sup>77</sup> SCFAs, as butyrate and propionate, play a key role in regulating the intestinal immune homeostasis with multiple actions on barrier function and have an anti-inflammatory effect.<sup>53,78</sup>

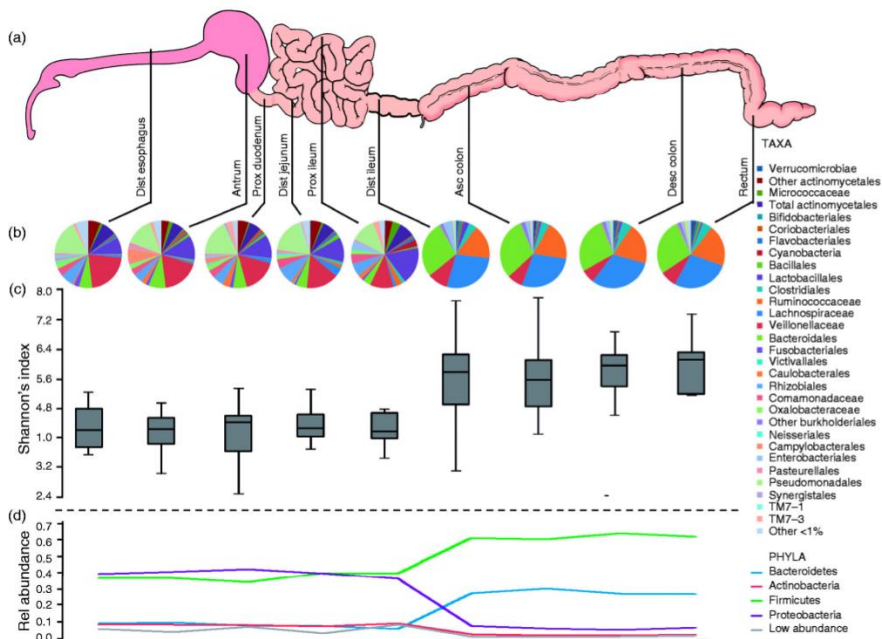
#### 1.2.1.1 The gut microbiota along the gastrointestinal tract

The composition of the microbiota varies through the gastrointestinal tract with alpha diversity (the microbial community variation within a sample) steadily increasing along the gastrointestinal tract. Hence, the lowest level of microbial diversity is seen from the oesophagus to the proximal part of the ileum; the highest level, from the terminal ileum to the rectum.<sup>79</sup> Most microorganisms in the human gut are mainly anaerobic, and these microorganisms belong primarily to the phyla *Bacteroidetes*, *Firmicutes* and *Proteobacteria*. Bacteria with minor representation in the healthy gut

(typically lower than 1%) belong to the phyla *Actinobacteria*, *Verrucomicrobia*, *Acidobacteria* and *Fusobacteria*.<sup>80</sup>

The mucosa-associated microbiota of the upper gastrointestinal tract is primarily consisting of *Proteobacteria* and *Firmicutes*. In the lower gastrointestinal tract, the number of *Proteobacteria* decreases, whereas the number of *Firmicutes* increases in the large intestine and has the highest number in the distal colon. *Bacteroidetes* is underrepresented in the upper gastrointestinal tract but dominates the lower gastrointestinal tract.<sup>79</sup> The most predominant bacterial families in the upper gastrointestinal tract, above antrum, are *Veillonellaceae*, *Pseudomonadaceae* and *Streptococcaceae*, whereas *Prevotellaceae* and *Helicobacteraceae* are dominant in the antrum. In the distal jejunum, *Bradyrhizobiaceae* is more prevalent than in other locations in the gastrointestinal tract, and *Micrococcaceae* is mainly found in the proximal ileum. The lower gastrointestinal tract is primarily consisting of *Lachnospiraceae*, *Bacteroidaceae*, *Ruminococcaceae* and *Veillonellaceae* (Figure 5).<sup>79</sup>

In addition to longitudinal variation of the gut microbiota, the composition of the microbiota also differs between the gut mucosa and gut lumen. Most bacteria are prevented from penetrating the mucus layer due to mucins produced by the goblet cells. Only specialised bacteria can adhere to the mucus, penetrate the mucus barrier, and access the epithelial cells. These bacteria include *Clostridium*, *Lactobacillus* and *Enterococcus*.<sup>81</sup> In contrast to the microbial composition in gut mucosa, the composition of the microbiota found in faeces harbours many different bacterial species, which can belong to *Bacteroides*, *Bifidobacterium*, *Streptococcus*, *Enterobacteriaceae*, *Enterococcus*, *Clostridium*, *Lactobacillus* and *Ruminococcus*.<sup>82</sup>



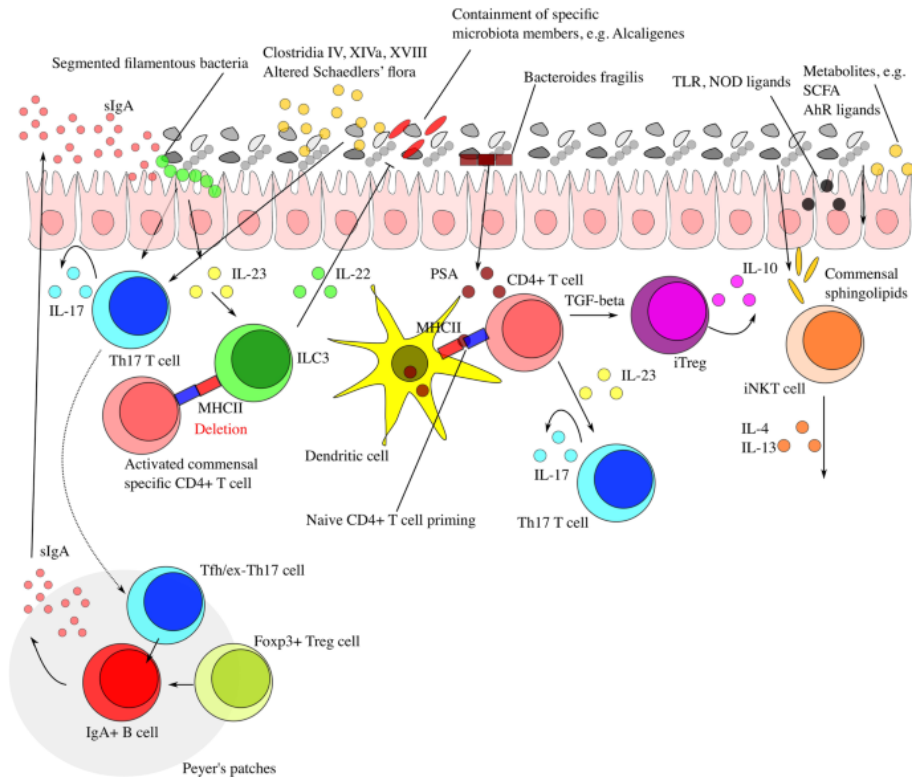
**Figure 5.** Gut microbiota along the gastrointestinal tract. Reprinted by permission from SAGE Publishing Company, from *Vuik FER, United European Gastroenterology Journal 2019 May;7:897–907. doi: 10.1177/2050640619852255.*

### 1.2.1.2 Interaction between the gut microbiota and the immune system

The microbiota interacts with the intestinal mucosa to establish an immune tolerance towards the commensal microbial microorganisms whilst preserving an immune response against pathogenic infection.<sup>83</sup> In healthy individuals, the immune response to the intestinal microorganisms is compartmentalised to the mucosal surface.<sup>84</sup>

The gut microbiota communicates with the innate immune system to maintain intestinal homeostasis. This interaction is facilitated by several mechanisms such as antimicrobial peptides produced by Paneth cells<sup>85</sup> and pattern recognition receptors like TLRs and NOD-like receptors (NOD1/NOD2).<sup>86–88</sup> These mechanisms have several functions as recognition of microbial signals during infection and causing a protective immune response, adjusting the abundance of commensal microbes and preserving mucosal tissue integrity. Together with the commensal microbiota, monocytes and macrophages function as crucial innate immune effector cells in maintaining homeostasis.<sup>89</sup> The phenotypic diversity and function of the intestinal innate lymphoid cells are influenced by signals from the gut microbiota.<sup>90</sup> Innate lymphoid cells are specialised in secretion of cytokines and chemokines in the infection control and promotion of mucosal tissue repair.<sup>91</sup>

In addition to the role of the microbiota in the innate immune response, the microbiota also interacts with the adaptive immune system. The B cells produce secretory immunoglobulin A (IgA) antibodies responsive to the commensal microbiota to preserve gut homeostasis.<sup>92</sup> It is found that a diversified and selected intestinal IgA repertoire helps to maintain a diversified and balanced microbiota.<sup>93</sup> Intestinal secretory IgA antibodies mainly coat colitogenic bacteria and prevent changes of the intestinal homeostasis and inhibit inflammation.<sup>94</sup> Furthermore, reactivity of intestinal and systemic CD4+ T cells to intestinal bacteria may reinforce homeostasis by causing the appearance of several immune cells protective against pathogens.<sup>95</sup> The gut microbiota can also regulate the adoptive T cell response involving CD8+ (cytotoxic) T cells in the elimination of intracellular pathogens. In this regulation, microbiota-derived SCFAs can advance the memory potential of antigen-activated CD8+ T cells.<sup>96</sup>



**Figure 6.** Microbiota-immune system interactions. Interactions between the microbiota (upper part of the figure) and the mucosal immune system (lower part of the figure) are illustrated with connecting arrows. AhR, aryl hydrocarbon receptor; Ig, immunoglobulin; IL, interleukin; iNKT cell, invariant natural killer T cell; iTreg, induced regulatory T cells; MHC, major histocompatibility complex; NOD, nucleotide oligomerization domain; PSA, polysaccharide A; SCFA, short-chain fatty acid; Tfh, T follicular helper cells; TGF-beta, transforming growth factor beta; Th, T helper cells; TLR, toll-like receptor. Reprinted by permission from Springer Nature, from Zheng D, *Cell Research* 2020 May;30:492–506. doi:10.1038/s41422-020-0332-7.

### 1.2.2. MICROBIOTA IN GASTROINTESTINAL DISEASES

Imbalanced homeostasis of the gut microbiota community, often termed dysbiosis,<sup>97</sup> is considered a possible contributor to development of gastrointestinal diseases such as IBD.<sup>98</sup> Dysbiosis of the gut microbiota is found when the diversity, composition

and/or functions of the gut microbiota are disrupted.<sup>98</sup> This can cause altered gut homeostasis and an inappropriate activation of the immune response.

Metagenomic studies indicate that the richness and diversity of certain bacterial species in the human gut may be an indicator of health.<sup>99</sup> Bacterial taxa associated with health benefits include *Bacteroides*, *Bifidobacterium*, *Clostridium* clusters XIVa/IV and *Lactobacillus* with association to enhanced metabolism, function of the immune system, cancer resistance, strengthened endocrine signalling and brain function.<sup>72,100</sup> The gut microbiota is quite resilient, but can be disrupted by antibiotic use, travel and illness. The gut microbiota has the ability to recover from negative impacts; however, this resilience can be lost with long term perturbations, which possible can have health implications.<sup>101</sup>

### 1.2.2.1 General changes in the gut microbiota in patients with IBD

In general, patients with IBD have reduced microbial diversity of mainly *Firmicutes* and an increased level of *Proteobacteria* including *Enterobacteriaceae*, *Bilophila* and specific members of *Bacteroidetes*.<sup>102,103</sup> Loss of microbial diversity can influence important functions required for maintaining intestinal barrier integrity and adjusting the host immune response.<sup>102</sup> Another change is the increased level of mucolytic and pathogenic bacteria, resulting in degradation of the mucosal barrier allowing movement of pathogens into the underlying intestinal tissues.<sup>102</sup> These changes can potentially result in increased immune responses leading to inflammation of the gut. However, it remains unclear if the changes in the gut microbiota trigger the inflammation or the inflammation causes the changes seen in the gut microbiota.

### 1.2.2.2 Changes in gut microbiota after IPAA surgery

The creation of a pouch will change the composition of the gut microbiota in the terminal ileum, which will respond by developing the pouch microbiota where early changes are found within 2 months after surgery.<sup>104</sup>

The microbiota profile found in the terminal ileum will change to a pouch microbiota consisting of a microbiota profile resembling those found in the colon, where especially *Enterococcus* spp. and *Lactobacillus* spp., predominant in the ileum microbiota, are reduced over time.<sup>104</sup> Furthermore, the level of anaerobic and colon-predominant bacteria like *Clostridium coccoides*, *Clostridium leptum*, *Bacteroides fragilis* and *Atopobium* increases over time after pouch surgery.<sup>104</sup>

Studies of the profile of the pouch microbiota characterised by culture-based faecal samples found the most prevalent bacterial species to be *Veillonella*, *Enterobacter*, *Klebsiella*, *Staphylococcus*, *Corynebacterium*, *Peptococcus*, *Clostridium* and *Lactobacillus*.<sup>105</sup>

### 1.2.2.3 Changes in gut microbiota in patients with pouchitis

As in patients with UC, the bacterial diversity is reduced in patients with pouchitis.<sup>106-108</sup>

An increased relative abundance of bacteria suggested to have a pathogenic potential is found in patients with pouchitis. Mucin-degrading bacteria like *Clostridium perfringens*, *Ruminococcus gnavus* and *Akkermansia muciniphila* have been associated with pouchitis.<sup>106,109,110</sup> Invasive bacteria capable of producing potentially toxic metabolic products like *Fusobacteria* and *Escherichia coli* have also been associated with pouchitis.<sup>111-113</sup>

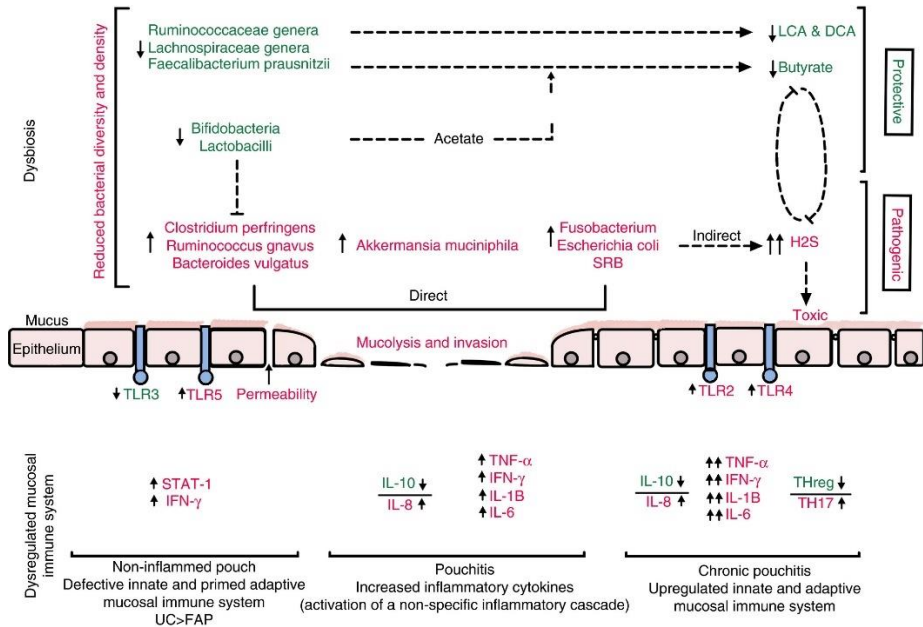
A decreased relative abundance of bacteria suggested to have a protective potential is found in patients with pouchitis. Studies have found a decreased relative abundance of butyrate-producing bacteria like *Faecalibacterium prausnitzii* and several genera of the families *Lachnospiraceae* and *Ruminococcaceae* in patients with a history of pouchitis or with active pouchitis compared with non-inflamed IPAA.<sup>108,112,114,115</sup> The *Bifidobacterium* and *Lactobacillus* species with the potential to suppress the growth of potentially pathogenic bacteria and increase the production of butyrate have been shown to be reduced in active pouchitis.<sup>116</sup>

In active pouchitis, faecal samples and biopsies show reduced numbers of *Bacteroidetes*,<sup>45,110,117</sup> *Enterococcaceae*,<sup>45,118</sup> *Lachnospiraceae*,<sup>108</sup> *Faecalibacterium* spp.,<sup>108</sup> *Ruminococcaceae*,<sup>112</sup> *Streptococci* spp.,<sup>111,112</sup> *Alcaligenaceae*<sup>112</sup> and *Bifidobacterium* spp.,<sup>116</sup> and increased numbers of *Enterobacteriaceae*,<sup>112</sup> including *Escherichia coli*,<sup>112</sup> *Fusobacterium*<sup>111</sup> and *Clostridia* spp.<sup>110,116</sup> A consistent finding is an increase in *Clostridium* species and a decrease in *Enterococcaceae*. Differences in the microbial composition between faecal and mucosal samples in patients with pouchitis have not been investigated.

In chronic pouchitis, faecal samples show reduced numbers of *Enterococcus* spp.,<sup>119</sup> *Faecalibacterium prausnitzii*,<sup>120</sup> *Clostridium* spp.,<sup>120</sup> *Ruminococcus* spp.,<sup>120</sup> *Eubacterium* spp.,<sup>120</sup> *Lachnospiraceae*<sup>120</sup> and *Insertae Sedis XIV*.<sup>120</sup> In biopsies, both *Streptococcus* spp. and *Clostridium* spp. were reduced.<sup>111</sup>

Finally, alterations of various microbial metabolic by-products are found in patients with pouchitis. The faecal concentration of SCFA, particularly butyrate, seems to be reduced in patients with pouchitis.<sup>121</sup> The faecal hydrogen sulphide concentration correlates positively with the severity of pouchitis and in patients with a recent pouchitis event.<sup>122</sup>





**Figure 7.** Pouch dysbiosis and dysregulated pouch mucosal immune system. Changes in the pouch microbiota in case of dysbiosis are illustrated in the upper part of the figure, and changes in the dysregulated mucosal immune system according to the type of pouch inflammation are illustrated in the lower part. DCA, deoxycholic acid; H<sub>2</sub>S, hydrogen sulphide; IFN- $\gamma$ , interferon gamma; IL, interleukin; LCA, lithocholic acid; SRB, sulphate-reducing bacteria; STAT-1, signal transducers and activators of transcription; TH reg, regulatory T cells, TLR, toll-like receptor; TNF- $\alpha$ , tumour necrosis factor alpha; ----->, promote; -----I, inhibit. Green-coloured texts refer to potentially beneficial components. Red-coloured texts refer to potentially pathogenic components. Reprinted by permission from John Wiley and Sons, from *Ardalan ZS, Aliment Pharmacol Ther. 2020 Oct;52:1323-1340. doi: 10.1111/apt.16085.*

### 1.2.3. NEXT-GENERATION SEQUENCING

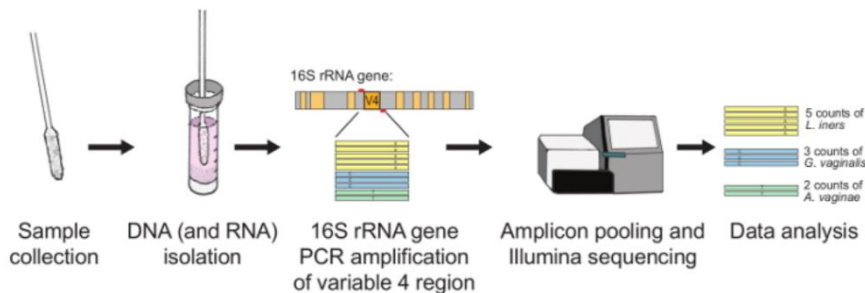
Culture-based methods remain important in clinical microbiology. However, when investigating the entire gut microbiota, culture-based methods have several shortcomings including bias towards bacteria that grow under laboratory conditions.<sup>123</sup> Molecular diagnostic techniques including polymerase chain reaction (PCR), DNA fingerprinting and next-generation sequencing (NGS) have emerged, becoming increasingly fast, sensitive and cost-efficient.<sup>124</sup> Therefore, NGS is now the first choice when analysing the gut microbiota. NGS includes the two main approaches for analysing the microbiome; 16S ribosomal RNA (16S-rRNA) gene

amplicon sequencing and whole genome shotgun (WGS) sequencing (shotgun metagenomic sequencing).<sup>124</sup>

### 1.2.3.1 16S-rRNA gene amplicon sequencing

The 16S rRNA amplicon sequencing technique is a marker gene-based approach, based on the amplification of small fragments of one or two hypervariable regions of the 16S rRNA gene.<sup>125</sup> The 16S rRNA gene is about 1,600 base pairs long and have nine hypervariable regions of varying conservation (region V1-V9). Among the hypervariable regions, more conservative regions is used to determine the higher-ranking taxa, and more rapidly evolving regions is used to identify genus or species.<sup>126</sup> Often semi-conserved hypervariable regions (primarily the V4 region) are selected as they can identify phylum level as precisely as the complete 16S gene.<sup>127</sup>

The workflow for NGS of 16S rRNA gene is DNA extraction; then amplification of the selected region of 16S rRNA gene using PCR and sequencing; and then classification of the sequenced data based on their similarity with reference 16S rRNA gene sequences found in public databases.<sup>128</sup> In general, the results from 16S rRNA amplicon sequencing can be used to evaluate microbial diversity down to genus and family levels. The resolution is normally insufficient to evaluate the species level.<sup>129</sup>



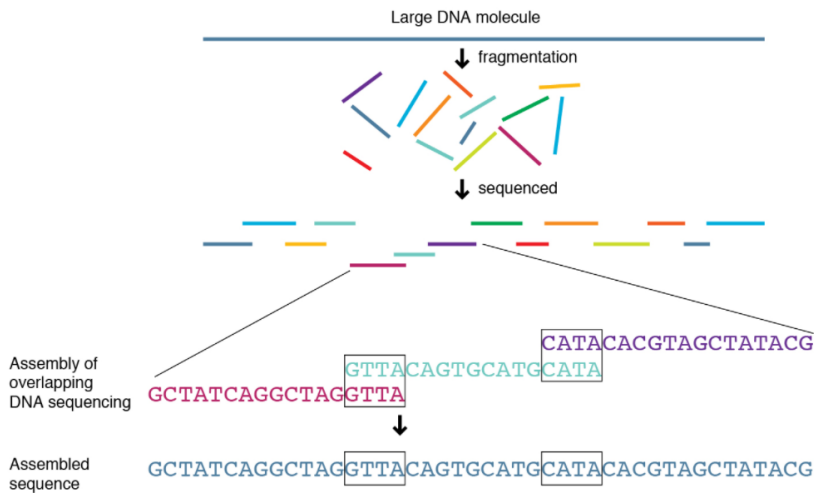
**Figure 8.** 16S rRNA amplicon sequencing technique. Reprinted by permission from JoVE, from *Anahitar MN, J. Vis. Exp. 2016;110:e53939. doi: 10.3791/53939.*

### 1.2.3.2 Whole genome shotgun sequencing (shotgun metagenomic sequencing)

Another sequencing technique beside 16S rRNA amplicon sequencing is WGS, which uses sequencing with random primers to sequence overlapping genome regions.<sup>124</sup> Shotgun metagenomic sequencing sequences all the genomic DNA from a given sample, and do not only target 16S rRNA genes.<sup>124</sup> This makes it possible to characterise the complete diversity of a habitat, including archaea, bacteria, eukaryotes, viruses and plasmids together with the gene content. Importantly, the

WGS sequencing method can also be used to describe the functional potential of the identified microorganism.<sup>130</sup>

Metagenomic shotgun sequencing is an unbiased sequencing method that discovers pre-fragmented DNA base pairs, which are randomly scattered like the pattern of a shotgun. The reads will either be generated from taxonomically regions like the 16S region or coding sequences. This to get information about the biological functions encoded in the genome in order to describe the biodiversity and function of a microbial community.<sup>130</sup>



**Figure 9.** Metagenomic shotgun sequencing technique. Reprinted by permission from National Human Genome Research Institute.

#### 1.2.4. FAECAL MICROBIOTA TRANSPLANTATION

Faecal microbiota transplantation (FMT) is the administration of stool from a healthy faecal donor into the intestinal tract of a patient to alter the composition of the patient's gut microbiota to achieve a health benefit.<sup>103</sup>

The first known description of using stool as therapy was described by Ge Hong in fourth-century China using stool called “yellow soup” for the treatment of different conditions including diarrhoea.<sup>131</sup> In 1958, Eiseman and colleagues described treatment with faecal enemas for pseudomembranous colitis, a treatment previously used in horses, introducing FMT into modern medicine.<sup>132</sup>

A key element in FMT is selection of a suitable donor. Faecal donors are screened according to international guidelines. Screening includes a health questionnaire and blood and faecal screening.<sup>133,134</sup> The health questionnaire is used to select a donor without a history of diseases like autoimmune, metabolic and malignant diseases. Stool sample screening is conducted to trace any potential pathogens. The faecal donor screening programme is dynamic with inclusion of more screening parameters, as recently a SARS-CoV-2 RT PCR stool test.<sup>135,136</sup> Faecal donors are selected based on the screening programme only; yet several studies have investigated if certain donors are more suitable than others, popularly termed ‘super-donor’. Such super-donors are recruited based on the engraftment potential of the donor’s microbiota or clinical efficacy.<sup>137</sup> However, no consistent results on selection of a super-donor have yet been presented.

When the faecal donor is approved for donation, the stool is prepared by mixing with water or saline, followed by filtration of the mixture to remove any food elements. Finally, glycerol is added as cryoprotection.<sup>138</sup> The faecal mixture is finally processed according to the FMT administration procedure (scope/tube, enema, capsules). Most often, the faecal mixture is frozen and stored at -80°C for later use, but it can also be administered fresh.<sup>139</sup> In several countries, stool banks have emerged making FMT more accessible for patients.<sup>133,140,141</sup>

The faecal mixture for FMT can be administered through several routes including a nasogastric tube, nasojejunal tube, esophagogastroduodenoscopy, colonoscopy, enema or capsules. One FMT treatment is normally clinically effective for recurrent *Clostridioides difficile* infection (rCDI); however, for UC, repeated FMT treatments are often needed to achieve a clinical effect. Typically, one faecal donor is selected for a patient. This restriction is applied primarily in regard to safety issues and traceability. However, multi-donor FMT has been used in clinical trials.<sup>142,143</sup> Finally, pre-treatment with antibiotics before FMT has been suggested to enhance microbiota engraftment and clinical efficacy, but studies investigating antibiotic pre-treatment have found mixed results.<sup>144,145</sup>

Most clinical experience with FMT comes from FMT for rCDI, where FMT is now a well-established treatment option in patients with rCDI. Furthermore, FMT is being tested in several clinical trials for treatment of other gastrointestinal diseases like IBD.<sup>146</sup>

#### **1.2.4.1 FMT for treatment of rCDI**

*Clostridioides difficile* infection is often precipitated by antibiotic treatment resulting in a subsequent change in the gut microbiota. For rCDI, the initial infection episode is treated but is followed by recurrence of diarrhoea and a positive stool test within 8 weeks.<sup>147,148</sup> Many patients with rCDI will often develop further recurrences, making management of these patients difficult. Studies comparing FMT against either

standard care antibiotics or placebo/autologous FMT have found incredible positive results.<sup>147,148</sup> Meta-analyses of 45 studies with FMT for treatment of patients with rCDI including nine randomised controlled trials (RCTs) found clinical efficacy at 84% with a single FMT and at 91% with repeated FMTs for rCDI.<sup>147,148</sup> FMT to rCDI is now a well-established treatment described in international therapeutic guidelines as a robust evidence based treatment option for rCDI.<sup>149-151</sup>

#### 1.2.4.2 FMT for treatment of IBD

Several clinical trials have tested the use of FMT in patients with IBD, showing substantial evidence for the use of FMT to induce clinical remission in mild to moderate UC. Multiple RCTs have shown positive results and convincing benefits over placebo. Pooled results from six RCTs in 324 patients with UC found that FMT was significantly superior to placebo in inducing clinical and endoscopic remission (OR 4.11, 95% CI 2.19-7.72;  $p < 0.0001$ ).<sup>152</sup> Long-term clinical remission after FMT is often lacking for several patients with UC, as many patients have disease relapse a median of 6 months after FMT.<sup>153</sup> However, the role of maintenance FMT therapy in patients with UC has been investigated. One RCT of 61 patients found comparable rates of clinical remission (87% vs 67%,  $p = 0.11$ ), but more patients were in endoscopic and histologic remission at week 48 after FMT delivered by colonoscopy every 8 weeks compared with patients treated with placebo.<sup>154</sup> In another RCT, ten patients with a clinical or endoscopic response after FMT were assigned to a maintenance phase. Patients were either treated with continue open-label FMT or withdraw therapy; all patients treated with continued FMT were in clinical, endoscopic and histological remission at week 56, and none of the patients who had withdrawn therapy were in remission.<sup>155</sup>

One placebo-controlled RCT tested the role of FMT in maintaining remission in CD with 17 patients included in the intention-to-treat analysis.<sup>156</sup> The study found no statistically significant clinical difference though there was a trend towards improved steroid-free remission at 10 weeks (88% vs 44%) and 24 weeks (50% vs 33%) for patients treated with FMT compared with placebo. Interpretation of case series and cohort studies of FMT to CD is limited by heterogeneous methods and results. Systematic reviews of cohort studies report an approximately 50% clinical remission rate after FMT in patients with CD.<sup>157-159</sup>



## CHAPTER 2. AIMS AND SCOPE

The influence of the gut microbiota on chronic pouchitis and the use of FMT as a new treatment option are investigated in the studies included in the thesis.

Research on FMT for treatment of patients with chronic pouchitis is limited and presents varying results. The use of FMT in UC and especially rCDI is well described, but solid evidence for its use in other diseases is lacking. Furthermore, the role of the gut microbiota in the pathogenesis of gastrointestinal inflammatory diseases is only partly understood. Therefore, the influence of the gut microbiota needs further evaluation in patients with chronic pouchitis.

To address this, the aims of the PhD thesis were:

- 1) To investigate differences in gut microbiota composition of faecal samples between inflamed and non-inflamed IPAAAs and healthy controls.
- 2) To conduct a systematic review of FMT for treatment of patients with chronic pouchitis. To summarise the results from the studies previously performed.
- 3) To conduct an open-label pilot study to investigate the use of multi-donor FMT for treatment of patients with chronic pouchitis.
- 4) To conduct an RCT investigating if non-pooled multi-donor FMT is superior to placebo in inducing clinical remission in patients with chronic pouchitis.





# CHAPTER 3. METHODS

For each of the aims mentioned above, the methods will be described in this chapter. First, a description of the study population for each of the clinical studies is given, followed by a description of the screening of faecal donors, the production of FMT material and the trial design for the FMT intervention studies. Second, a description of the literature study is given. Third, a description is presented of microbiome sequencing of the collected faecal samples from the patients, donors and healthy individuals. Fourth, the data handling, including statistical analyses and ethics, will be described.

## 3.1. STUDY POPULATIONS

This thesis is primarily based on clinical trials on patients with chronic pouchitis and their controls including faecal donors. Participants were included in the following three studies: Study I, III and IV.

In Study I, participants were recruited from November 2017 to June 2019 at the Department of Gastrointestinal Surgery, Aalborg University Hospital, Aalborg, Denmark. Included patients had either a normally functioning pouch, chronic pouchitis or FAP. Healthy controls were also included. A normally functioning pouch after IPAA surgery for UC was defined as no episodes of pouchitis, no symptoms of pouch dysfunction or no antibiotics use for pouchitis (during the past year). Chronic pouchitis was defined as  $\geq 3$  episodes of pouchitis with a PDAI score  $\geq 7$  during the past year. Patients with FAP had surgical removal of the large intestine due to the FAP diagnosis with subsequent no history of pouchitis or use of antibiotics (during the past year). Healthy controls with no history of bowel diseases were recruited from the Blood Bank at Aalborg University Hospital, Aalborg, Denmark. All the participants delivered a faecal sample and completed a questionnaire including stool frequency, antibiotic use and pouch function for patients with an IPAA.<sup>160</sup>

In Study III, patients with chronic pouchitis were recruited from May 2018 to October 2018 at the Department of Gastrointestinal Surgery, Aalborg University Hospital, Aalborg, Denmark. Patients were  $\geq 18$  years and had their IPAA for  $>1$  year. Chronic pouchitis was defined as  $\geq 3$  episodes of pouchitis during the past year. The patients should have a clinical PDAI score  $\geq 3$  points and had been treated with ciprofloxacin and/or metronidazole  $\geq 1$  time during the past year. Patients were excluded in case of immunosuppression, pregnancy or breastfeeding, or positive faecal test for enteric bacterial pathogens.<sup>161</sup>

In Study IV, patients with chronic pouchitis were recruited from October 2019 to January 2022 at either the Department of Gastrointestinal Surgery, Aalborg University Hospital, Aalborg; the Department of Hepatology and Gastroenterology, Aarhus University Hospital, Aarhus; the Department of Gastroenterology and Hepatology, Odense University Hospital, Odense; or the Gastroint, Hvidovre Hospital, Copenhagen University Hospital, Hvidovre, Denmark. Included patients should be  $\geq 18$  years with an IPAA for  $>1$  year. Chronic pouchitis was defined as  $\geq 3$  episodes of pouchitis during the past year and/or pouchitis with continued symptoms  $>4$  weeks despite treatment with antibiotics. Patients had a total PDAI score  $\geq 7$  point, a clinical PDAI score  $\geq 3$  points and were treated with ciprofloxacin and/or metronidazole  $\geq 1$  time during the past 3 months. Patients were excluded in case of immunosuppression, pregnancy or breastfeeding, positive faecal test for enteric bacterial pathogens, serious food allergy and/or previous anaphylactic reaction.

## 3.2. FAECAL DONOR SCREENING AND PREPARATION OF FMT MATERIAL

FMT material was prepared from faecal donors for FMT treatment of patients with chronic pouchitis in Study III and IV.

### 3.2.1. FAECAL DONOR SCREENING

In Study III and IV, faecal donors were recruited from the Blood Bank at Aalborg University Hospital, Aalborg, Denmark. Healthcare personnel employed at the hospital were not excluded as donors. Included healthy individuals were screened according to international guidelines for FMT using a healthy screener questionnaire, and blood and faecal tests were obtained before and after faecal donation for one month.<sup>134,161</sup>

Faecal and blood screening included a faecal test for intestinal pathogenic bacteria viruses, parasites, multi-resistant microorganisms, *Helicobacter pylori*, faecal calprotectin and SARS-CoV-2 (during the COVID-19 pandemic). Blood test included a general health screening with haemoglobin, erythrocytes, thrombocytes, leukocytes, C-reactive protein, creatinine, alanine aminotransferase, lactate dehydrogenase, albumin, alkaline phosphatase, bilirubin and HbA1c, and screening for cytomegalovirus, Epstein-Barr virus, human immunodeficiency virus, hepatitis and syphilis.<sup>161</sup>

Once approved by screening, faecal donors entered the 1-month faecal donation period. During this period, faecal donors were instructed to maintain their normal healthy lifestyle, avoid travelling outside of Denmark and avoid any other behaviours associated with acquisition of communicable diseases.<sup>161</sup>

Five faecal donors were approved by screening and donated stool to the FMT treatment in Study III; 13, in Study IV.<sup>161</sup>

### **3.2.2. FMT MATERIAL PREPARATION**

The donated faecal samples for FMT treatment were handled according to the international consensus conference article on stool banking.<sup>133</sup>

The faecal donors were equipped with a cooling box and freezer packs. Immediately after producing stool for donation, the donor placed the stool in the colling box surrounded by frozen freezer packs. The stool was delivered within 3 hours after defecation at the Department of Gastrointestinal Surgery, Aalborg University Hospital with a delivery note. Thereafter, the stool was processed in the laboratory at the Department of Clinical Microbiology, Aalborg University Hospital. Stool was divided into portions of 20 grams of faeces (for Study III) and 50 grams of faeces (for Study IV). For each portion, 100 ml of sterile water was added and homogenised manually using a blender (Braun MQ 325). It was subsequently filtered through two layers of sterile gauze or a filtration Seward Stomacher® Bag. The filtrated sample was mixed with glycerol (100% dissolution) to a final concentration of 10% glycerol for freeze protection. The final mixture was poured into a sterile enema bottle of 100 ml mixture per bottle. The enema bottles were stored at -80°C at latest four hours after delivery. Each enema bottle contained stool from one faecal donor only. The enema bottles were quarantined after production and released to FMT treatment only once the donor had passed the second screening.<sup>161</sup>

### **3.3. FMT TRIAL DESIGN**

Study III was a prospective, open-label, single-centre cohort pilot study.<sup>161</sup> Nine of the ten included patients completed the 14 days of FMT treatment with enema from the five faecal donors, each daily enema from a single donor. Any treatment with antibiotics and probiotics was stopped seven days prior to FMT. The first FMT was administered at the outpatient clinic at the Department of Gastrointestinal Surgery, Aalborg University Hospital, and patients were instructed to perform the remaining FMTs at home the following 13 days. During the treatment period, patients recorded daily changes in cPDAI and stool frequency, and they recorded any adverse events during the treatment period. At inclusion and at the 30-day follow-up, patients underwent a pouchoscopy without bowel cleansing; biopsies and faecal samples were collected; and the complete PDAI was assessed. Faecal samples were collected and cPDAI, stool frequency and adverse events were recorded monthly during a 6-month follow-up period.<sup>161</sup>

Study IV was a multi-centre, randomised placebo-controlled study. The 30 included patients were randomised at a block randomisation ratio of 1:1 to treatment with either non-pooled multi-donor FMT delivered by enema from four faecal donors (5-6 enema bottles from each of the four faecal donors) or placebo (water-based mixture with glycerol and food colouring). Patients were treated once daily for 14 consecutive days followed by treatment every second day for 14 days. Patients and outcome assessors were blinded for the type of treatment. Any treatment with antibiotics or probiotics was stopped before project treatment. The first treatment was administered at the outpatient clinic, and the patients performed the remaining treatments at home. On a daily basis during the treatment period, patients recorded cPDAI, stool frequency and any adverse events, and collected four faecal samples. At inclusion and at the 30-day follow-up, patients had a pouchoscopy and biopsies and faecal samples were collected, and the complete PDAI was assessed. The primary outcome of clinical remission in the FMT and placebo group was assessed at the 30-day follow-up. Patients dropping out of the study due to treatment failure were invited to receive open-label multi-donor FMT if requested.

### 3.4. LITERATURE STUDY

In Study II, a literature search was completed in Medline, EMBASE and the Cochrane Central Register of Controlled Trials Library on 15 April 2020.<sup>162</sup> Additional eligible studies were found by searching bibliographies of review articles, the primary author of included studies and Web of Science. Results from unpublished studies were found in Clinicaltrials.gov and on the WHO International Clinical Trials Registry Platform, and opengrey.eu was used to search for grey literature.<sup>162</sup>

Eligible studies were defined as human interventional studies using FMT for treatment of chronic pouchitis (recurrent and antibiotic-refractory chronic pouchitis) describing changes of pouch symptoms. All types of studies were allowed, including conference abstracts. In controlled studies, the accepted control arm was placebo, autologous FMT or no treatment. Studies should be written in/or translated into English. Studies where chronic pouchitis was not the primary condition treated with FMT were excluded.<sup>162</sup>

Clinical response was defined as a reduction in PDAI  $\geq 3$ . Clinical remission was defined as a reduction in PDAI  $\geq 3$  and total PDAI  $< 7$ . Data reported in the mPDAI and cPDAI were accepted. Follow-up assessment of clinical changes in the PDAI score at any timepoint after FMT was accepted.<sup>162</sup> Risk of bias and quality assessment of the included studies were evaluated using the Cochrane risk of bias tool for RCTs,<sup>163</sup> and the US National Heart, Lung, and Blood Institute quality assessment tool was used for cohort and case studies.<sup>164</sup>

Study II was performed in accordance with the PRISMA 2009 guidelines.<sup>165</sup> A research protocol was submitted to Prospero International Prospective Registry of Systematic Reviews (registration number: CRD42020167258).

### **3.5. NEXT-GENERATION SEQUENCING OF THE GUT MICROBIOTA**

The composition of the gut microbiota in faecal samples collected from patients and healthy individuals including faecal donors in Study I, III and IV was assessed using NGS.

#### **3.5.1. 16S RRNA AMPLICON SEQUENCING**

16S rRNA amplicon sequencing was used in Study I and III to analyse the microbiome in collected faecal samples.<sup>160,161</sup> All faecal samples were initially stored in a biobank at -80 °C. DNA extraction from faecal samples was performed using QIAamp PowerFecal DNA Kit from QIAGEN. Bacterial microbiota profiling using the hypervariable V4-region of the 16S rRNA gene was used to analyse the composition of the gut microbiota in faecal samples.<sup>160,161</sup> The 16S rRNA amplicon sequencing was conducted at the Department of Chemistry and Bioscience, Section for Bioscience and Engineering, Aalborg University, Aalborg.

#### **3.5.2. SHOTGUN METAGENOMIC SEQUENCING**

Shotgun metagenomic sequencing was used in Study IV to analyse the gut microbiota in collected faecal samples. All the faecal samples were initially stored in a biobank at -80 °C. DNA was extracted from the faecal samples using DNeasy® 96 Powersoil® Pro QIAcube HT kit with a slightly modified protocol. Shotgun metagenomic sequencing of the faecal samples was conducted at the Department of Chemistry and Bioscience, Section for Bioscience and Engineering, Aalborg University, Aalborg, using the NovaSeq Illumina platform. Samples were sequenced to a depth of a median of 4.5 Gb.

### **3.6. STATISTICS**

Data were analysed in either STATA® V.17.0 (StataCorp LP, Texas, USA) or R v. 3.6.0 through Rstudio v. 1.1.383 (<http://www.rstudio.com>). A P-value <0.05 was considered statistically significant.

In Study I,<sup>160</sup> community richness was calculated using the observed number of amplicon sequence variants (ASVs), and diversity was calculated with the Shannon Diversity Index. Beta diversity was investigated using principal component analysis (PCA) on Hellinger-transformed ASV abundances. The R packages ampvis2, tidyverse and vegan were used.<sup>166–168</sup> Permutation tests of pairwise linear regression were used to evaluate the statistical significance of the groupings in PCA using the pairwise.factorfit function from the RVAideMemoire package.<sup>169</sup> Wilcoxon rank sum test was used for statistical comparison, and Holm p-value correction was selected to address multiple testing.<sup>170</sup>

In Study II,<sup>162</sup> pooled estimates for clinical response and remission were analysed for all patients treated with FMT in the included studies. The baseline characteristics were presented in a table with count for discrete variables and continuous variables presented with mean and range. Analyses were performed according to the intention-to-treat principle, with dropouts defined as treatment failures.

In Study III,<sup>161</sup> the Wilcoxon signed-rank test compared clinical and biological variables across groups, and a paired Wilcoxon signed-rank test compared samples from the same patient at inclusion and the 30-day follow-up. Community richness was analysed using an observed number of ASVs, and diversity was analysed using the Shannon Diversity Index. Beta diversity was investigated using PCA on Hellinger-transformed ASV abundances. The R packages ampvis2, vegan, data.table, ggplot and tidyr were used.<sup>166,171–174</sup> Similarity of samples was investigated using the Sørensen–Dice coefficient,<sup>175</sup> and testing for differential abundance by using DESeq2 with Benjamini–Hochberg-adjusted p-values.<sup>176</sup>

In Study IV, baseline characteristics were presented in a table with count and percentage or mean and standard deviation (SD). The primary outcome compared clinical remission (either total PDAI <7 or reduction in PDAI  $\geq$ 3 points together with total PDAI <7) at the 30-day follow-up between treatment groups using a modified Poisson regression with robust variance estimator to calculate the relative risk (RR) with 95% CI. The Poisson regression with identity link function was used to calculate the risk difference (RD) of the primary outcome. Changes in the total PDAI score, PDAI sub-scores and stool frequency were evaluated using a linear regression model. The daily cPDAI score and stool frequency were presented in figures with separate point-wise confidence intervals for FMT and placebo. Differences in baseline parameters between patients in remission compared with patients in relapse at the 30-day follow-up were analysed using Student's t-test. Differences in patients achieving remission between patients with a history of repeated antibiotics compared with those being treated with continuous antibiotics were analysed using Fisher's exact test. Adverse events reported during treatment were presented in a table with count and percentage. All analyses were performed according to the intention-to-treat principle. Missing outcome results at the 30-day follow-up were evaluated as treatment failure if it was due to dropout and rescue treatment with antibiotics. Microbiota data analysis

was performed with the R packages tidyverse,<sup>167</sup> vegan,<sup>177</sup> ggplot2,<sup>173</sup> ggpubr<sup>178</sup> and ampvis2.<sup>166</sup> Microbial richness was calculated as species with relative abundance above zero, and alpha diversity was calculated using the Shannon Diversity Index. Similarity with donors was assessed with the Sørensen coefficient and Bray-Curtis similarity on relative abundances and Hellinger-transformed abundances, respectively. Beta-diversity was further investigated using PCA and redundancy analysis (RDA) on Hellinger-transformed relative abundances constrained by time and treatment type. Paired and unpaired Wilcoxon rank sum test was used to calculate differences before/after treatment and between patients/donors.

All bioinformatics in Study I, III and IV were performed by researchers with great experience in bioinformatics at the Department of Chemistry and Bioscience, Section for Bioscience and Engineering, Aalborg University, Aalborg.

### **3.7. ETHICAL CONSIDERATIONS**

The clinical studies were performed in adherent to the requirements of Good Clinical Practice and the Revised Declaration of Helsinki. All patients included in the clinical studies gave signed written informed consent to participate. The Regional Research Ethics Committee of Northern Jutland, Denmark approved the three clinical studies (project number Study I N-20180013, Study III N-20180008 and Study IV N-20150021).

The two FMT studies were registered at ClinicalTrials.gov (trial number Study III NCT03538366 and Study IV NCT04100291).





# CHAPTER 4. RESULTS

This section gives a summary of the results from the studies performed.<sup>160-162</sup> Study I-III are published articles and Study IV is a draft article.

## 4.1. STUDY I: THE GUT MICROBIOTA PROFILE IN PATIENTS WITH INFLAMED AND NON-INFLAMED ILEAL POUCH-ANAL ANASTOMOSIS

### 4.1.1. PARTICIPANTS

In total, 38 participants were included, 11 patients with a normally functioning pouch, 9 patients with chronic pouchitis, 6 patients with FAP and 12 healthy controls (HCs) were included. Characteristics of the included participants are presented in Table 4.<sup>160</sup>

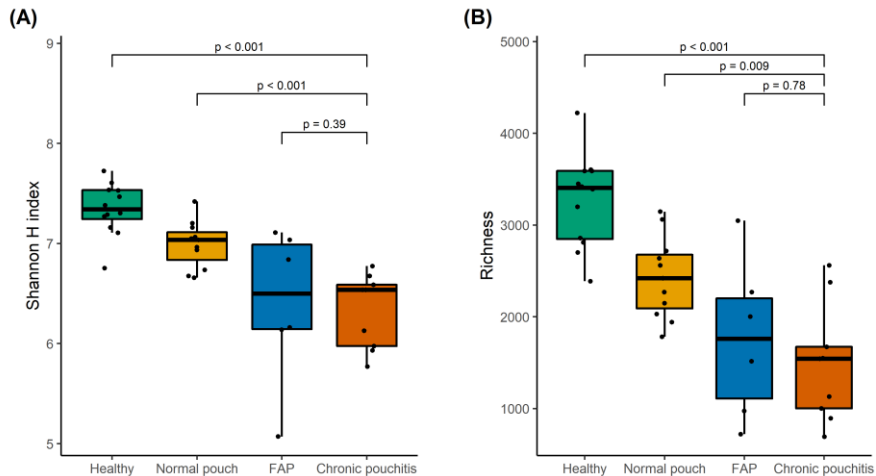
Groups	Normal pouch function	Chronic pouchitis	FAP	HCs
Age <i>mean</i> (SD)	47.1 (11.0)	52.9 (13.7)	54.8 (16.3)	42.3 (13.9)
Male <i>n</i> (%)	7 (64)	3 (33)	1 (17)	8 (69)
Time since surgery <i>mean years</i> (range)	12.9 (5–21)	17.6 (8–28)	14.2 (3–30)	-
Stool frequency <i>mean</i> (range)	5.5 (3–8)	11.2 (5–20)	5.5 (1–8)	1.2 (1–2)
cPDAI <i>mean</i> (range)	0.6 (0–1)	3.7 (3–5)	1.0 (1–1)	-
Continues antibiotic use <i>n</i> (%)	0 (0)	3 (33)	0 (0)	0 (0)

**Table 4.** Characteristics of patients with a normally functioning pouch, chronic pouchitis or familial adenomatous polyposis and healthy controls. cPDAI, clinical Pouchitis Disease Activity Index; FAP, familial adenomatous polyposis; HCs, healthy controls; n, number; SD, standard deviation.

### 4.1.2. ANALYSIS OF THE GUT MICROBIOTA

- Lower microbial diversity and richness were described in faecal samples from patients with chronic pouchitis compared with patients with a normally functioning pouch ( $p < 0.001$  and  $p = 0.009$ ) and HCs ( $p < 0.001$  and  $p < 0.001$ ) (Figure 10).<sup>160</sup>

- No difference in diversity or richness between patients with chronic pouchitis and patients with FAP was observed ( $p=0.39$  and  $p=0.78$ ) (Figure 10).<sup>160</sup>



**Figure 10.** The Shannon Diversity Index (A) and the number of amplicon sequencing variants (ASVs) for species richness (B) in patients with a normally functioning pouch, familial adenomatous polyposis (FAP) or chronic pouchitis and healthy individuals. Reprinted by permission from MDPI, from Kousgaard J, S *et al. Microorganisms* 2020;8(10):1611. <https://doi.org/10.3390/microorganisms8101611>.

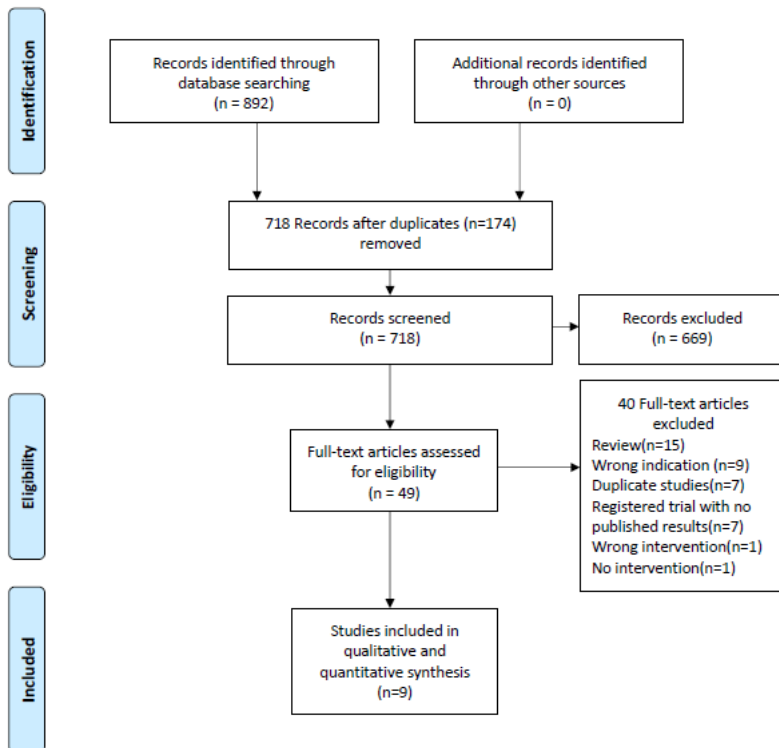
- Patients with chronic pouchitis had an overall altered composition of the gut microbiota than patients with a normally functioning pouch and HCs.<sup>160</sup>
- The most abundant genera in patients with a normally functioning pouch and HCs were genus *Bacteroides* or genus *Prevotella* (Figure 11A).<sup>160</sup>
- The most abundant genera in patients with chronic pouchitis and FAP were genus *Bacteroides* for all except two chronic pouchitis patients who both received continuous antibiotic treatment (Figure 11A).<sup>160</sup>



## 4.2. STUDY II: FAECAL MICROBIOTA TRANSPLANTATION IN TREATMENT OF CHRONIC POUCHITIS – A SYSTEMATIC REVIEW

### 4.2.1. STUDY SELECTION

The literature search found 892 studies. After exclusion of duplicates, screening and full-text review, nine studies were found eligible for inclusion (Figure 12).<sup>162</sup>



**Figure 12.** Flowchart of review and selection of studies for systematic review. Reprinted by permission from MDPI, from *Cold, F and Kousgaard J, S et al. Microorganisms* 2020;18(9):1433. <https://doi.org/10.3390/microorganisms8091433>.

## 4.2.2. PATIENT CHARACTERISTICS

In the nine included studies, 65 patients were treated with FMT (Table 5). The majority of the studies were case series/reports or small pilot studies, and only one was a RCT study.<sup>162</sup>

Characteristics	
Number of studies, <i>n</i>	9
Number of patients, <i>n</i>	65
Number of FMT treatments in days, <i>mean</i> (range)	4.8 (1–14)
Delivered grams of stool by FMT, <i>mean</i> (range) ( <i>n</i> = 51)	111.8 (11–525)
Days of follow-up, <i>mean</i> (range) ( <i>n</i> = 65)	87.6 (28–365)
Male/female, <i>n</i> ( <i>n</i> = 51)	22/29
Age of patients, <i>mean</i> (range) ( <i>n</i> = 51)	43.8 (22–77)
Years since restorative proctocolectomy, <i>mean</i> (range) ( <i>n</i> = 50)	10.3 (1–33)
FMTs with single/multi-donor, <i>n</i> ( <i>n</i> = 65)	56/9
FMTs with related/unrelated faecal donor, <i>n</i> ( <i>n</i> = 65)	12/53
FMTs by upper/lower/both administration, <i>n</i> ( <i>n</i> = 51)	13/32/6
Number of patients in RCT/non-RCT, <i>n</i> ( <i>n</i> = 65)	6/59

**Table 5.** Characteristics of patients from the included studies in the systematic review. FMT, faecal microbiota transplantation; *n*, number; RCT, randomised controlled trial.

## 4.2.3. FMT FOR TREATMENT OF CHRONIC POUCHITIS

- In patients for whom changes in the PDAI score were assessed, clinical response was achieved in 14/44 patients (31.8%) and clinical remission was achieved in 10/44 patients (22.7%) at various timepoints after FMT.<sup>162</sup>
- In the one RCT, none of the six treated patients achieved clinical remission.<sup>179</sup>
- Small reductions in the endoscopic and histologic PDAI scores were found in two studies,<sup>161,180</sup> while no or minimal changes were reported in two studies.<sup>181,182</sup>

- In two studies, a decrease in faecal calprotectin was found in patients with a reduced symptom/PDAI score,<sup>180,183</sup> while two studies found no or a minimal decrease.<sup>179,182</sup>
- The studies were very heterogeneous in terms of donor selection, stool processing, treatment length and delivery, scoring of treatment efficacy and follow-up.<sup>162</sup>
- Several studies reported minor self-limiting adverse events, mainly abdominal pain, nausea, bloating, fever, dizziness, fatigue and feeling uncomfortable.<sup>161,180-182</sup>

#### 4.2.4. GUT MICROBIOTA

- Six studies investigated bacterial alpha diversity in faecal or mucosal samples,<sup>162</sup> with five studies finding no significant changes.<sup>161,179,181,182,184</sup>
- Steube et al. showed a significantly increased bacterial alpha diversity in patients who had clinical improvement after FMT.<sup>183</sup>
- Seven studies investigated engraftment of the donor microbiota in recipients after FMT.<sup>161,179-184</sup> In three studies, resemblance to donors' microbiota increased after FMT and correlated with a beneficial clinical effect.<sup>179,180,182</sup>
- Four studies investigated changes in the relative abundance of certain bacteria species after FMT.<sup>180-183</sup> Bacterial species like family *Ruminococcaceae* and *Lachnospiraceae* and genus *Faecalibacterium* were increased and *Escherichia coli* decreased in abundance in patients after FMT.

### 4.3. STUDY III: CLINICAL RESULTS AND MICROBIOTA CHANGES AFTER FAECAL MICROBIOTA TRANSPLANTATION FOR CHRONIC POUCHITIS – A PILOT STUDY

#### 4.3.1. PATIENT POPULATION

Nine of the ten included patients with chronic pouchitis received and completed FMT treatment. The characteristics of the nine patients are illustrated in Table 6.<sup>161</sup>

Characteristics	FMT (n = 9)
Age mean (SD)	51.5 (13.9)
BMI kg/m <sup>2</sup> mean (SD)	25.3 (5.7)
Male n (%)	3 (33.3)
Years since IPAA surgery mean (SD)	17.6 (6.7)
Continuous antibiotic use n (%)	3 (33.3)
Anti-diarrhoea drug use n (%)	7 (77.8)

**Table 6.** Characteristics of patients with chronic pouchitis treated with faecal microbiota transplantation. BMI, Body Mass Index; n, number; SD, standard deviation.

#### 4.3.2. CLINICAL OUTCOMES

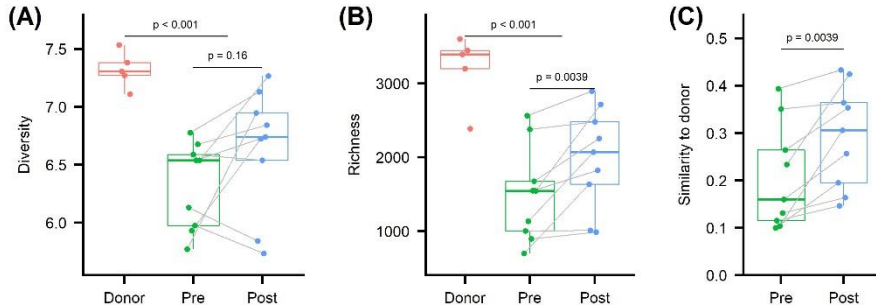
- Four of the nine patients achieved clinical remission at the 30-day follow-up, and three patients were still in remission at the 6 months follow-up. Relapsing patients relapsed between 0-52 days after FMT treatment.<sup>161</sup>
- The PDAI score did not improve significantly between inclusion (mean 8.6, SD 3.4) and the 30-day follow-up (mean 5.2, SD 4.5).<sup>161</sup>
- The cPDAI score decreased statistically significantly from mean 3.7 (SD 0.7) at inclusion to mean 1.6 (SD 1.7) at the 14-day follow-up (p=0.02). The mean cPDAI score was 2.0 (SD 1.7) at the 30-day follow-up.<sup>161</sup>
- The endoscopic and histologic PDAI score improved from inclusion to the 30-day follow-up, but the decrease was not statistically significant.<sup>161</sup>
- Stool frequency decreased from mean 11.2 daily bowel movements at inclusion to 10.4 at the 30-day follow-up, and faecal calprotectin decreased from 732.1 µg/g at inclusion to 152 µg/g at the 30-day follow-up.<sup>161</sup>

#### 4.3.3. ADVERSE EVENTS

- Seven patients reported one or several adverse events during FMT treatment, all being minor self-limited events with abdominal discomfort or pain as the most frequently reported events.<sup>161</sup>

#### 4.3.4. GUT MICROBIOTA

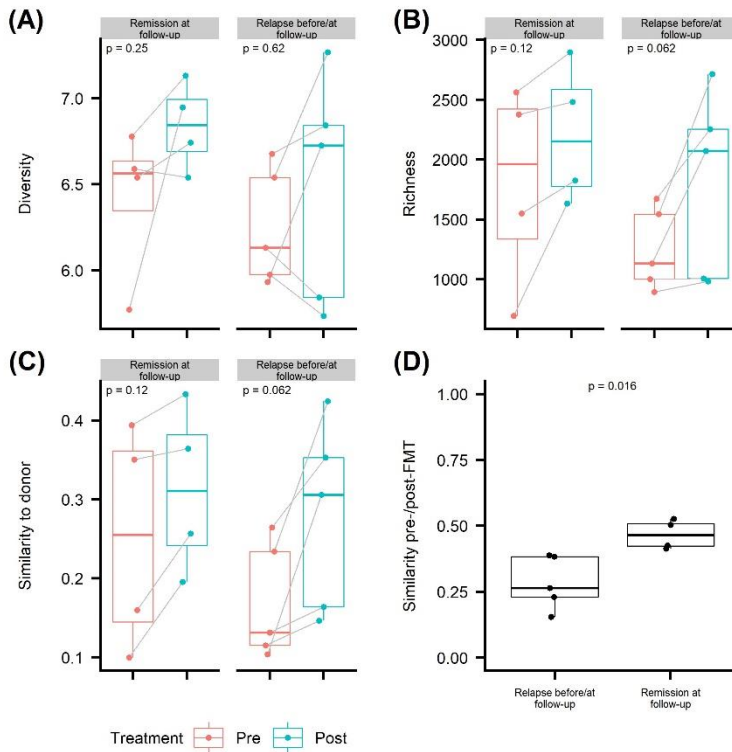
- After FMT treatment, microbial richness and marginal diversity increased in patient samples from inclusion to the 30-day follow-up ( $p=0.004$  and  $p=0.16$ , respectively) (Figure 13A;B).<sup>161</sup>
- A higher similarity between patients and donors was found after FMT ( $p=0.004$ ) (Figure 13C).<sup>161</sup>



**Figure 13.** The Shannon Diversity Index (A) and number of amplicon sequencing variants (ASVs) for species richness (B) in patients with chronic pouchitis and faecal donors. (C) Similarity to donors for patient samples before and after FMT. Reprinted by permission from Taylor & Francis, from *Kousgaard J, S et al. Scand J Gastroenterol. 2020;55(4):421-429. doi: 10.1080/00365521.2020.1748221.*

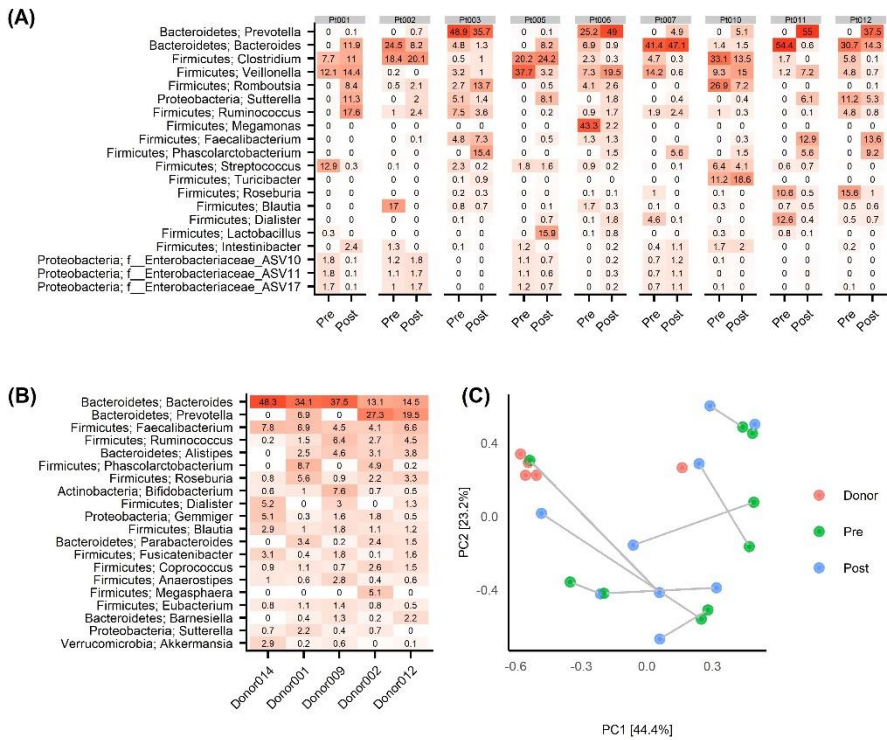
- Patients in remission at the 30-day follow-up had a more resilient microbiota with higher similar microbial composition before/after FMT than patient who relapsed ( $p=0.016$ ) (Figure 14D).<sup>161</sup>





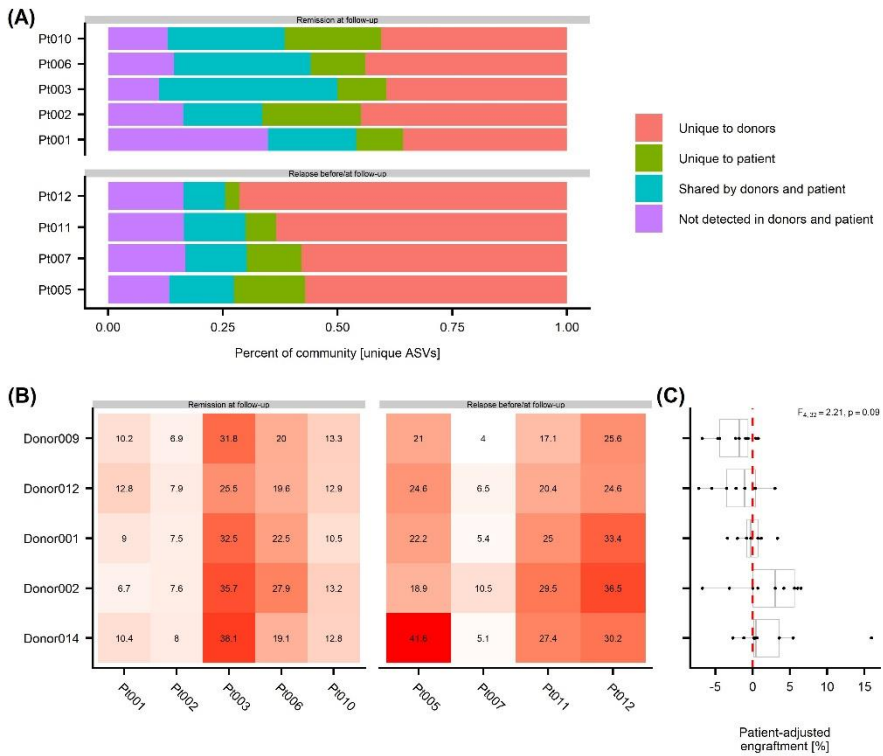
**Figure 14.** Microbiota community characteristics in patients, stratified by remission/relapse at the 30-day follow-up. The Shannon Diversity Index (A) and number of amplicon sequencing variants (ASVs) for species richness (B) for patients stratified by remission/relapse. The similarity to donors for patients before and after FMT split by relapse (C). The similarity of samples from patient after FMT with the corresponding FMT sample from the same patient before FMT (D). Reprinted by permission from Taylor & Francis, from *Kousgaard J, S et al. Scand J Gastroenterol. 2020;55(4):421-429. doi: 10.1080/00365521.2020.1748221.*

- The relative abundance of genera *Ruminococcus* and *Bacteroides* was more pronounced in faecal samples from the donors than from patients (Figure 15A;B).<sup>161</sup>
- Donor and patient samples could be separated in the PCA plot ( $p=0.02$ ; Figure 15C), but not patient samples before/after FMT.<sup>161</sup>



**Figure 15.** Microbial composition of patients with chronic pouchitis and faecal donors. Relative abundance with phylum names given for patients (A) and donors (B). A principal component analysis (PCA) plot of the first two components for all faecal samples from patients and donors (C). Reprinted by permission from Taylor & Francis, from *Kousgaard J, S et al. Scand J Gastroenterol. 2020;55(4):421-429. doi: 10.1080/00365521.2020.1748221.*

- A high engraftment of ASVs unique to the donor microbiota in patients' faecal samples after FMT.<sup>161</sup>
- Comparing engraftment between patients in remission and patients in relapse; several donor ASVs were engrafted (p=0.016) and fewer AVSs were shared between the donors and patients (p=0.016) (Figure 16A).<sup>161</sup>
- A successful engraftment of donor microbiota was both patient specific (p<0.001) and borderline donor specific (p=0.09).<sup>161</sup>



**Figure 16.** Engraftment of donor microbiota community after faecal microbiota transplantation (FMT) in patients with chronic pouchitis. Percentage microbiota community after FMT that is unique to the donors, unique to the patient, existing in both donors and patient and unobserved in community before FMT (A). Cross-tabulation of the percentage of ASVs in each patient on the x-axis which is also existing in individual donors on the y-axis (B). The donor-specific engraftment of donor microbiota community (C). Reprinted by permission from Taylor & Francis, from Kousgaard J, S et al. *Scand J Gastroenterol.* 2020;55(4):421-429. doi: 10.1080/00365521.2020.1748221.

#### 4.4. STUDY IV: NON-POOLED MULTI-DONOR FAECAL MICROBIOTA TRANSPLANTATION TO INDUCE CLINICAL REMISSION IN PATIENTS WITH CHRONIC POUCHITIS: A RANDOMISED PLACEBO-CONTROLLED TRIAL

##### 4.4.1. PATIENT POPULATION

A total of 68 patients with chronic pouchitis were assessed for eligibility between September 2019 and January 2022; 30 were randomised; 15 to FMT and 15 to placebo. Four participants dropped out of the study during the treatment period (three FMT group, one placebo group) due to treatment failure. During treatment, five patients missed one or several treatments (1-8 treatments). In total, 26 patients completed the 30-day assessment. Baseline patient demographics are illustrated in Table 7.

After dropping out of the study, 11 patients were treated with open-label FMT in the open-label extension FMT study.

Characteristic	Faecal microbiota transplantation (n = 15)	Placebo (n = 15)
Gender, n (%)		
Women	11 (73)	9 (60)
Men	4 (27)	6 (40)
Age, years, mean (SD)	45.9 (12.6)	52.4 (13.8)
BMI, kg/m <sup>2</sup> , mean (SD)	24.6 (4.8)	25.7 (4.3)
Years since IPAA surgery, mean (SD)	18.3 (7.2)	19.2 (8.5)
PDAI, mean (SD)	8.9 (1.8)	9.1 (1.3)
Stool frequency, mean (SD)	13.1 (4.4)	11.2 (2.7)
CRP, mg/L, mean (SD)	6.4 (5.8)	3.7 (3.7)
Leucocytes, x10 <sup>9</sup> /L, mean (SD)	7.5 (1.8)	7.2 (1.89)
Faecal calprotectin, µg/g, mean (SD)	337.4 (378.3)	479.5 (560.4)
Medication, n (%)		
Loperamide	5 (33)	8 (53)
Codeine	4 (27)	2 (13)
Other diseases besides pouchitis, n (%)		
Any disease	5 (33)	9 (60)

**Table 7.** Baseline characteristics of patients with chronic pouchitis randomised to faecal microbiota transplantation or placebo. BMI, Body Mass Index; CRP, C-reactive protein; PDAI, Pouchitis Disease Activity Index.

#### 4.4.2. PRIMARY OUTCOME

- Clinical remission at the 30-day follow-up was achieved equally in patients who received FMT and patients who received placebo (6/15 [40%] vs 6/15 [40%]; RD  $-3.89e^{-17}$  [95%CI -0.36-0.36]; RR 1 [95%CI 0.55-1.81];  $p=1.000$ ).
- Clinical remission and a reduction in PDAI score were achieved in 3/15 (20%) patients treated with FMT compared with 6/15 (40%) treated with placebo (RD 0.82; [95%CI 0.59-1.13]; RR 0.5 [95%CI 0.15-1.67];  $p=0.260$ ).

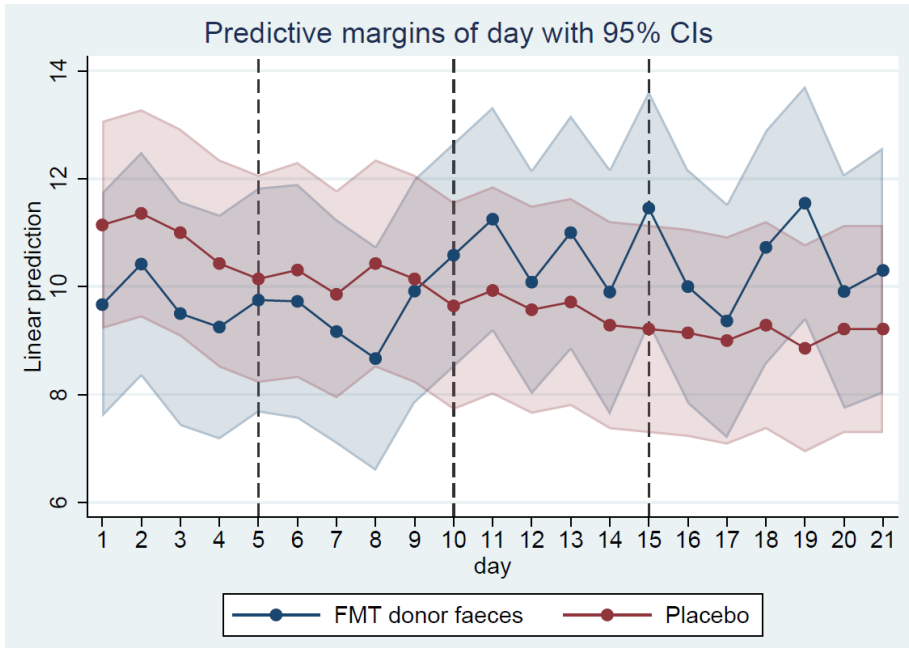
#### 4.4.3. SECONDARY OUTCOMES

- Clinical response at the 30-day follow-up was achieved in 4/15 (27%) patients who received FMT compared with 8/15 (53%) patients who received placebo (RD 0.77; [95%CI 0.54-1.08]; RR 0.5; [95%CI 0.19-1.33];  $p=0.166$ ).
- The mean total PDAI score decreased in both the FMT and placebo group at the 30-day follow-up (mean change in the FMT group 1.83 [SD 2.37]; in placebo group, 2.57 [SD 2.50]).
- No statistically significant differences were found in the primary and secondary outcomes between the FMT and the placebo group (Table 8); a trend towards a more prominent decrease in the placebo group than in the FMT group was seen only for cPDAI.

Outcome	Faecal microbiota transplantation (n = 12)		Placebo (n = 14)		P value
	Inclusion	30-day follow-up	Inclusion	30-day follow-up	
<b>Primary outcome</b>					
PDAI, mean (SD)	8.7 (1.7)	6.8 (2.5)	9.0 (1.2)	6.4 (2.7)	0.695
<b>Secondary outcomes</b>					
Stool frequency, mean (SD)	13.5 (4.8)	11.0 (3.4)	11.4 (2.6)	8.8 (3.9)	0.141
Faecal calprotectin, mean (SD)	273.8 (338.3)	345.4 (500.1)	489.1 (580.3)	441.1 (426.4)	0.603
cPDAI, mean (SD)	4.1 (1.0)	2.8 (1.3)	3.6 (0.6)	1.7 (1.3)	0.056
ePDAI, mean (SD)	3.0 (2.0)	3.3 (1.8)	3.8 (1.2)	3.6 (1.7)	0.645
hPDAI, mean (SD)	1.6 (0.7)	0.8 (0.6)	1.6 (0.6)	1.1 (0.8)	0.414

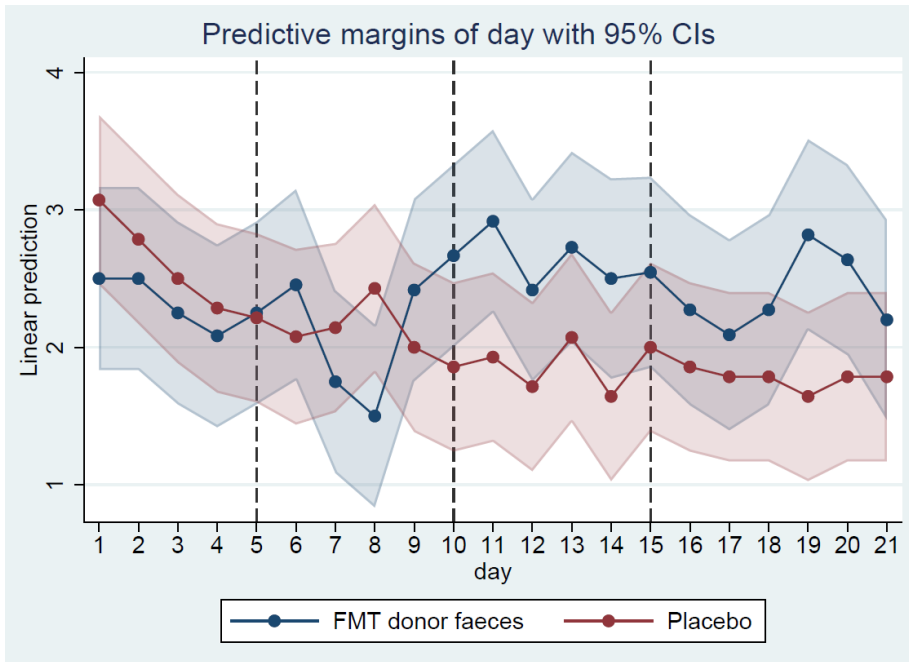
**Table 8.** Outcome measures comparing faecal microbiota transplantation with placebo at the 30-day follow-up. cPDAI, clinical Pouchitis Disease Activity Index; ePDAI, endoscopic Pouchitis Disease Activity Index; hPDAI, histologic Pouchitis Disease Activity Index; PDAI, Pouchitis Disease Activity Index.

- The mean stool frequency decreased in both the FMT and placebo group at the 30-day follow-up (mean change in the FMT group 2.50 [SD 3.90]; placebo group, 2.64 [SD 4.88]); difference 0.14 [95% CI -3.76-3.47],  $p=0.94$ ).
- Changes in daily stool frequency during the 4-week treatment with FMT/placebo are illustrated in Figure 17.



**Figure 17.** Changes in daily stool frequency during the 4-week treatment with faecal microbiota transplantation or placebo.

- The mean cPDAI score decreased in both the FMT and placebo group at the 30-day follow-up (mean change in the FMT group 1.33 [SD 1.50]; placebo group, 1.93 [SD 1.64]); difference 0.60 [95% CI -0.68-1.87],  $p=0.347$ ).
- Changes in daily cPDAI score during the 4-week treatment with FMT/placebo are illustrated in Figure 18.



**Figure 18.** Changes in daily clinical Pouchitis Disease Activity Index during the 4-week treatment with faecal microbiota transplantation or placebo.

- No statistical difference was found in baseline parameters between patients in remission compared with patients in relapse at the 30-day follow-up. Patients in remission had a non-significant lower disease activity at inclusion than relapsing patients (PDAI score 8.5 vs. 9.4,  $p=0.118$ , and faecal calprotectin 333  $\mu\text{g/g}$  vs. 459  $\mu\text{g/g}$ ,  $p=489$ ).
- Three of the 10 patients (30%) receiving continuous antibiotics were in remission at the 30-day follow-up compared with 9 of the 20 patients (45%) receiving repeated antibiotics ( $p=0.694$ ).

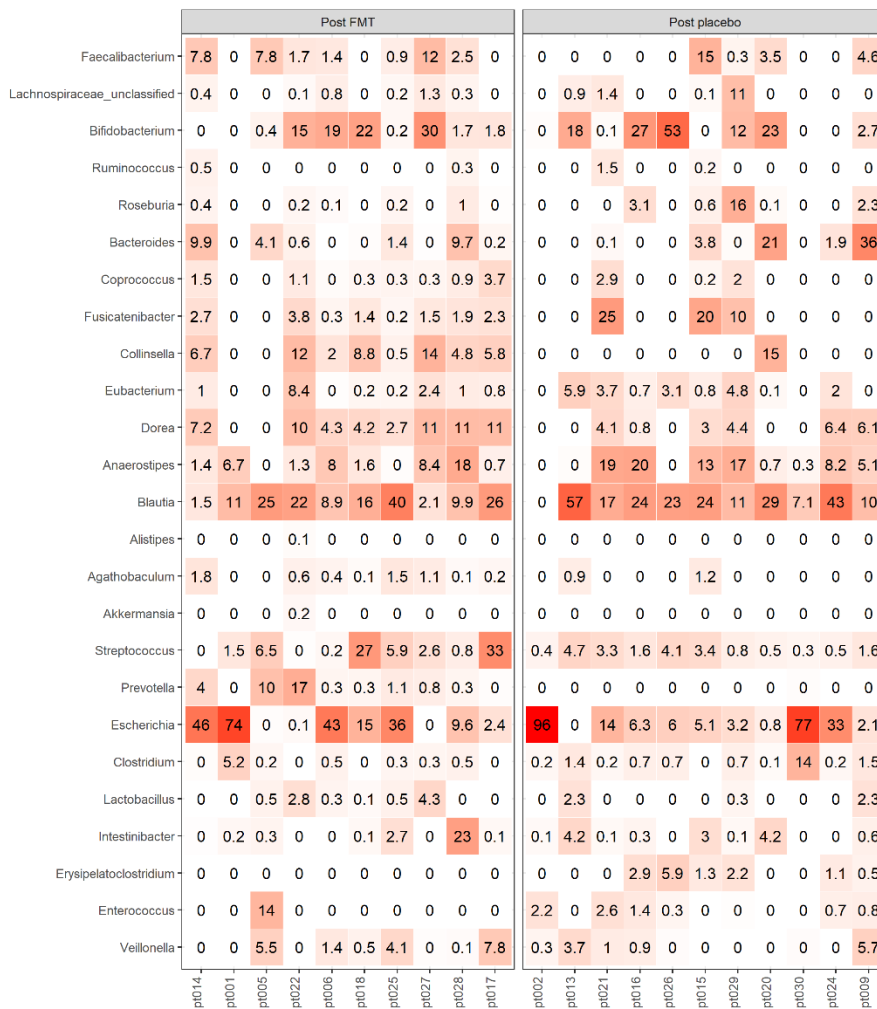
#### 4.4.4. GUT MICROBIOTA

- All but four donor batch samples were successfully sequenced and passed quality control. Two samples from the FMT group and three samples from the placebo group were discarded from the analysis after quality control. Therefore, the gut microbiota was assessed for 10 patients in the FMT group, 11 patients in the placebo group and all 13 faecal donors.
- Donor faecal samples had higher microbial diversity than patient faecal samples, regardless of treatment group.
- Using the weighted mean in Hellinger-transformed relative abundance analysis, we found that the microbial community of donor samples comprised more genera



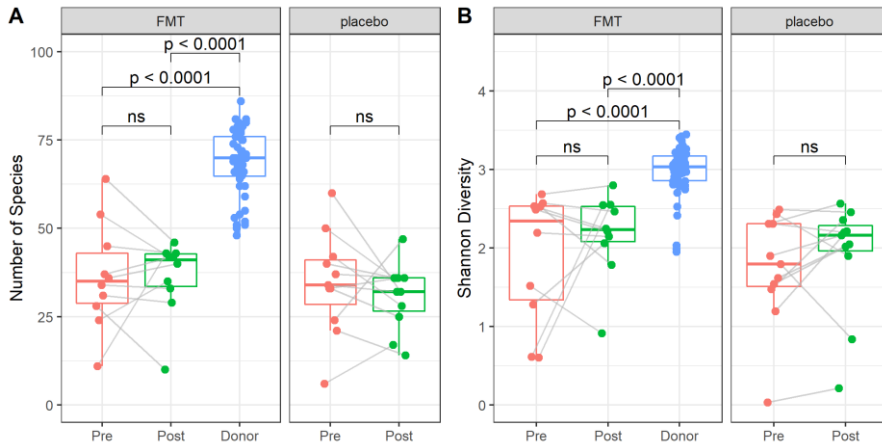


composition after FMT treatment did alter the microbiome slightly. Notably, the *Faecalibacterium* is found in four patients in the FMT group after treatment who did not have this genus at inclusion; *Escherichia*, which dominated the faecal microbiome in pt005 and pt022 at inclusion, has been eliminated. It is also noticed that there is a great difference in the microbial composition among patients both in the FMT and placebo group (Figure 20).



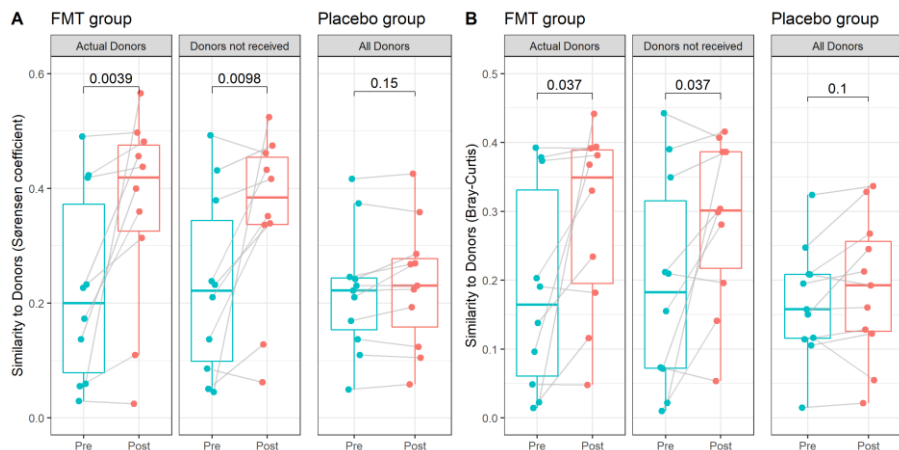
**Figure 20.** Microbial composition of faecal samples from patients with chronic pouchitis after treatment with faecal microbiota transplantation or placebo. The top 25 most abundant genera based on the weighted mean of Hellinger-transformed relative abundance for samples before FMT, before placebo, after FMT, after placebo and in donors. Numbers are relative abundance in per cent.

- The microbial alpha diversity of faecal samples from patients was significantly lower than the diversity in faecal samples from donors as measured by both species richness and Shannon diversity ( $p < 0.0001$ ) (Figure 21 A,B). FMT or placebo treatment did not significantly change the microbial richness or diversity for either treatment group ( $p \gg 0.05$ ).



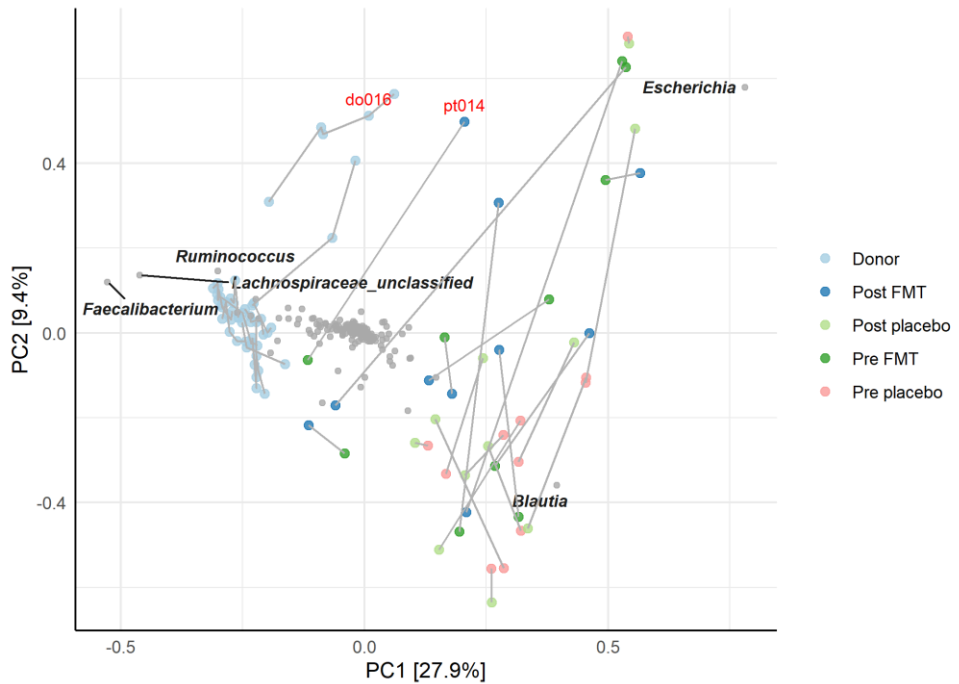
**Figure 21.** Microbial alpha diversity of pouchitis patient and donor faecal samples stratified by treatment. For patients, the number of species (A) and Shannon diversity (B) were calculated before and after treatment. Patients receiving FMT are plotted with donor batches used for treatment. P-values showing the pre- vs post-difference were analysed using paired Wilcoxon rank sum test, whereas p-values between patients and donors were analysed using unpaired Wilcoxon rank sum test. Each dot is a faecal sample. Faecal samples before and after treatment are connected by a line.

- Measuring beta-diversity by both the Sørensen coefficient and the Bray-Curtis similarity Hellinger-transformed relative abundances, FMT treatment did increase the median similarity with the faecal donor microbiome ( $p < 0.05$ ). Placebo treatment did not significantly increase the median similarity with all faecal donor microbiomes ( $p > 0.05$ ) (Figure 22 B).



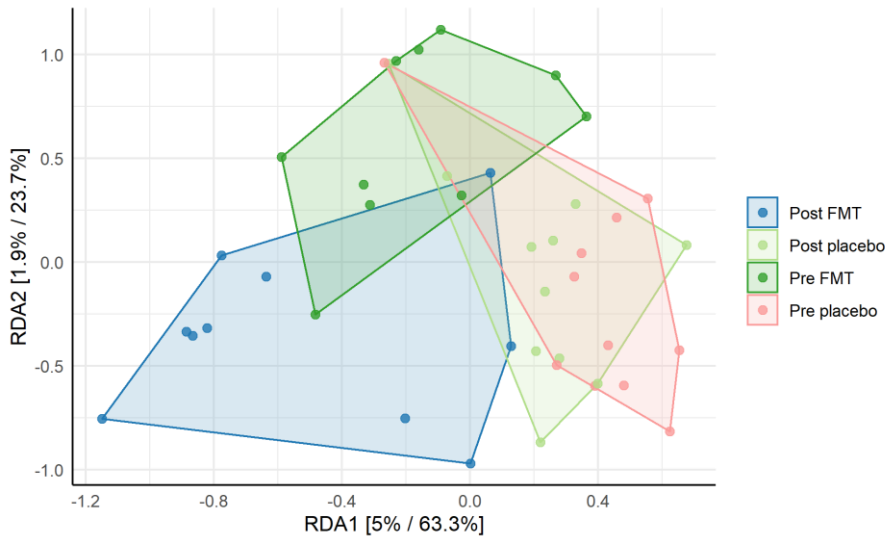
**Figure 22.** Median similarity of patient and donor microbiomes measured by Sørensen coefficient (A) and Bray-Curtis similarity on Hellinger-transformed relative abundances (B). The patients receiving FMT are compared with the faecal microbiomes received from donors (actual donors) and not received from donors (donors not received). The placebo group was compared to faecal samples from all donors (all donors). Every dot is a patient faecal sample. Faecal samples before and after treatment are connected by a line.

- PCA revealed a distinct donor cluster clearly separated from patient samples mainly driven by the relative abundance of three species from the genera *Faecalibacterium*, *Lachnospiraceae* and *Ruminococcus* (Figure 23). The patients' samples before treatment form two less distinct groups driven by two species from the genera *Escherichia* and *Blautia*. Though shifts in patients receiving placebo were observed, the shifts were in general not towards the donor group. Shifts towards the donor group are observed for five patients receiving FMT (shift from right-to-left). The faecal microbiome of one patient (pt014) clustering closely with the donor group pre-treatment shifted towards the last donor batch (do016) received by the patient (Figure 23).



**Figure 23.** Principal component analysis based on Hellinger-transformed relative abundance of donor batches and patient samples before and after treatment. Grey dots are species. The genus of the five most extreme species is named. Grey line connects patient samples or donor batches. Donor do016 and patient pt014 are shown in the plot.

- The effect of FMT compared with placebo treatment was assessed by RDA constrained by time and treatment (Figure 24). RDA revealed no differences before and after treatment with placebo, whereas a shift in microbial composition before and after FMT treatment can be observed. *Ad hoc* pairwise permutation analysis revealed no significant difference between before and after treatment for either treatment group.



**Figure 24.** Redundancy analysis of Hellinger-transformed relative abundance patient samples before and after treatment constrained by time (before, after) and treatment (placebo, FMT). The percentage of total variation and the percentage of total constrained variation explained by each axis is specified in the axis title.

#### 4.4.5. ADVERSE EVENTS

- In total 9 of the 30 patients had no adverse events, but 21 of the 30 patients (70%) reported one or several adverse events (1-14 adverse events per patient) during the study treatment, mainly abdominal pain or feeling discomfort (Table 9).
- In the FMT group, 12 of the 15 patients (80%) reported adverse events compared with 9 of the 15 (60%) patients in the placebo group.

Type of adverse event	Faecal microbiota transplantation	Placebo
Total number of patients with one or several adverse events, n (%)	12 (80)	9 (60)
Total number of all adverse events, n (%)	86 (100)	40 (100)
Abdominal pain, n (%)	25 (29)	6 (15)
Discomfort, n (%)	22 (26)	4 (10)
Diarrhoea, n (%)	14 (16)	6 (15)
Nausea, n (%)	11 (13)	1 (3)
Headache, n (%)	1 (1)	8 (20)
Itch or rash, n (%)	0	6 (15)
Bloated, n (%)	1 (1)	4 (10)
Fever, n (%)	3 (3)	0
Belch, n (%)	3 (3)	0
Sweating, n (%)	2 (2)	1 (3)
Others*, n (%)	4 (5)	4 (10)

**Table 9.** Adverse events reported during treatment in faecal microbiota transplantation and placebo group. The total number of all the adverse events reported in each treatment group is presented. Below, all the adverse events are arranged in groups. \*Adverse events each reported two times or less (joint pain, rectal bleeding, stomach rumbling, faecal incontinence, dizziness)

#### 4.4.6. FMT OPEN LABEL

- All 11 patients included in the FMT open-label extension study completed FMT treatment. Six of the 11 (55%) patients had a cPDAI score <3 at the 30-day follow.
- The mean cPDAI score decreased from 3.5 (SD 0.7) at baseline to 2.0 (SD 1.2) at the 30-day follow-up.
- The stool frequency decreased from mean 12.1 (SD 4.3) at baseline to mean 8.9 (SD 3.6) at the 30-day follow-up.
- The faecal calprotectin decreased from mean 406.0 µg/g (SD 422.0) at baseline to mean 357.3 µg/g (SD 327.6) at the 30-day follow-up.

## CHAPTER 5. DISCUSSION

The role of the gut microbiota in chronic pouchitis has been investigated throughout the studies included in this thesis, which has focused on the use of FMT as a new treatment option for chronic pouchitis. The gut microbiota appears to be altered in patients with chronic pouchitis compared with patients with a normally functioning IPAA and healthy individuals. FMT is not superior to placebo in inducing clinical remission in patients with chronic pouchitis, but a subgroup of patients did improve clinically after FMT.

### 5.1. COMPARISON WITH CURRENT LITERATURE

Previous studies of the composition of the gut microbiota in patients with pouchitis found that bacterial diversity is reduced compared with that of healthy individuals and patients with IPAA without inflammation.<sup>106</sup> Our results are therefore in line with those of previous studies. Furthermore, previous studies have found an increased relative abundance of potentially pathogenic bacteria like mucin-degrading bacteria, and a decrease in the relative abundance of potentially protective bacteria like butyrate-producing bacteria in patients with pouchitis.<sup>107</sup> In general, we found that for almost all participants, the main abundant genera were the genus *Bacteroides*, which is one of the most prevalent bacterial species in the human gut.<sup>74</sup> However, when we compared the composition of the gut microbiota between participants, we found that patients with chronic pouchitis had a bacterial composition very different from that of patients with a normally functioning IPAA and healthy individuals.<sup>160</sup> Notably, we found that the genus *Bacteroides* was mainly associated with healthy individuals, while the family *Enterobacteriaceae*, particularly the genus *Escherichia*, was predominant in patients with chronic pouchitis.<sup>160</sup> Finally, the composition of the gut microbiota in the chronic pouchitis samples was very heterogenous, which could be due to by the long-term nature of treatment with a variety of antibiotics.<sup>160</sup> In patients with FAP, the composition of the gut microbiota was also very heterogenous.<sup>160</sup> Previous studies investigating the composition of the gut microbiota in patients with chronic pouchitis were small and mainly culture based, which hampers comparison with our results.<sup>107</sup> In the studies based on 16S rRNA sequencing of faecal samples, Petersen et al. primarily found an increased abundance of *Proteobacteria* in patients with an IPAA.<sup>113</sup> This finding is comparable with our results which showed that the genus *Escherichia* in the phylum *Proteobacteria* was more abundant in patients with a pouch than in healthy individuals.<sup>160</sup> Like us, Zella et al. found that *Clostridium* was more abundant in patients with pouchitis than in patients with healthy pouches.<sup>110</sup> Furthermore, similar to us, Bálint et al. found a higher abundance of *Enterobacteriaceae* in patients with pouchitis than in healthy individuals.<sup>185</sup>

The use of FMT for treatment of chronic pouchitis has been reported in nine published studies including two RCTs,<sup>162,186</sup> where the use of FMT to induce clinical remission or improve clinical parameters has been investigated in 75 patients with chronic pouchitis.<sup>179–184,187–189</sup> These studies are very heterogeneous in terms of treatment duration and delivery, scoring of treatment efficacy and follow-up. Some of the studies found high clinical remission rates,<sup>180,183</sup> whereas others reported no or minor beneficial effect of FMT,<sup>181,182,188</sup> reporting remission rates in the range between 0% and 100%. The two RCTs found a clinical remission rate of 0% and 31%, respectively, after repeated FMT with faeces from a single donor; however, they reported no difference in remission rate when comparing FMT with placebo or autologous FMT.<sup>179,189</sup> In comparison, we found a remission rate of 44% and 40% in our two FMT trials, respectively.<sup>161</sup> This level of remission is comparable to rates reported in the studies that did report high remission rates.<sup>162</sup> Furthermore, in line with the two RCTs, we found no benefit of FMT compared with placebo; in addition, it seemed that patients receiving placebo experienced greater clinical improvement than patients receiving FMT.

Adverse events related to FMT have been described, among others abdominal pain, diarrhoea, and fever.<sup>190</sup> These adverse events can also be seen in the disease course of pouchitis, which hampers assessment of specific adverse events associated with FMT treatment in this patient group. In our RCT FMT trial, adverse events were more frequently reported for patients treated with FMT than for patients receiving placebo; this was especially the case for abdominal pain and diarrhoea, and this fact may have influenced the clinical scoring of disease activity in the pouch.

Unlike the studies previously reported by others, our FMT trials stand out as we used non-pooled multi-donor FMT and had a long treatment period.<sup>161</sup> Clinical trials have used pooled multi-donor FMT in cases with mild and moderate UC, finding multi-donor FMT to be significantly more successful in inducing clinical remission than placebo or autologous FMT.<sup>142,191</sup> Moreover, these studies found no difference in the number of reported adverse events between the two treatment groups. However, a systematic review of six RCT trials with FMT in patients with UC found no benefit of multi-donor FMT compared with single-donor FMT.<sup>152</sup> For rCDI, both single-donor and multi-donor FMT induce clinical remission rates in more than 80% of recipients.<sup>192</sup>

## 5.2. STRENGTHS

The main strength of the studies included in the present thesis is that, to our knowledge, the RCT FMT trial reported here is larger than previously published trials on FMT as a therapeutic modality in patients with chronic pouchitis. Furthermore, unlike several previously published trials, the international PDAI scoring system for



IPAA patients was used for all patients in our clinical trials. All patients with chronic pouchitis included in the clinical studies were evaluated with a complete PDAI score. Furthermore, all patients underwent pouchoscopy with collection of biopsies which were later histologically assessed.

Another strength is the use of repeated FMT. Thus, previous studies both on chronic pouchitis and UC indicate that more than one FMT is needed to induce clinical remission. Furthermore, unlike previous studies, we also used continuous FMT followed by a phasing-out period in the RCT with the purpose of hoping to achieve a long-term clinical effect. The use of enemas was chosen for FMT delivery as it was possible for patients to perform the procedure themselves at home, which was needed due to the long treatment period. Other FMT delivery methods have been used in patients with chronic pouchitis with no great difference in the clinical efficacy between studies.<sup>179–184,187–189</sup> A water-based mixture with glycerol and food colouring was used as the “placebo stool” in the RCT FMT trial. This is the standard approach customarily used in RCT trials not using autologous FMT. Furthermore, we used non-pooled multi-donor FMT where patients receiving FMT received FMT from different faecal donors over time. To maintain transferability, the donors’ stools were not pooled; instead, patients were treated with FMT from different donors on different days during the treatment period. The issue of how to select donors to achieve the best clinical effect in patients remains an unresolved issue.<sup>193</sup> However, multi-donor FMT may heighten the chance of finding the best donor match for patients. Moreover, the use of multi-donor FMT can increase microbiota diversity during treatment, and this may increase the chance that organisms contained in the transplanted faeces harbour potential to correct a functional deficit in patients’ gut microbiota.<sup>191</sup>

The faecal samples used for analysis of the gut microbiota were collected before and after study treatment, at every follow-up visit and during the treatment in the RCT FMT study. This makes it possible to investigate changes in the gut microbiota during and after treatment, and later at follow-up. Such data can be compared with clinically collected data. Microbiome sequencing was performed using standard sequencing techniques, and metagenomic sequencing was used in Study IV with the advantages of comprehensive sequencing of all DNA or RNA present in the sample. All microbiome sequencing was performed at a world-recognised lab for microbiome research using state-of-art techniques.

Lastly, our studies were performed in full accordance with the current international guidelines on donor screening and stool processing for FMT.<sup>133,141</sup> The systematic review on FMT for treatment of chronic pouchitis was performed in accordance with the PRISMA guideline.<sup>165</sup>

### 5.3. LIMITATIONS

Several limitations exist in the studies included in the thesis. These limitations are discussed below.

The first limitation is the sample size in the clinical studies. The clinical studies had small sample sizes; the RCT FMT trial did not include the planned number of patients; and a smaller proportion of patients failed to complete the study treatment. Therefore, the study was not sufficiently powered to allow us to draw definitive conclusions concerning the importance of the gut microbiota in pouchitis and the effectiveness of FMT for treatment of patients with chronic pouchitis. This is also clear especially in the study investigating the composition of the gut microbiota in different patient groups and healthy controls. Here, the sample sizes of the individual participant sub-groups were too small to allow us to accurately determine differences in the gut microbiota between groups. In general, large clinical studies on patients with chronic pouchitis are challenging to perform due to the limited number of patients available for inclusion. In Denmark, it is estimated that only about 500 patients have chronic pouchitis. Therefore, multinational studies are probably needed if the influence of the gut microbiota and the role of FMT in chronic pouchitis should be tested in the future.

The second limitation concerns the heterogeneity of the patients included in the clinical studies. Patients with chronic pouchitis are very heterogeneous, and both patients with different severity of clinical symptoms of pouchitis were included in our studies, which makes it difficult to fully compare patients. However, as chronic pouchitis is a rare disease, additional restriction of patients for inclusion will make it even more challenging to perform such a study. Furthermore, patients included in the clinical FMT studies were not pre-treated with antibiotics, which is a standard approach in FMT treatment of rCDI.<sup>150</sup> Patients with chronic pouchitis often have a long history of antibiotic treatments, which probably limits the effect of any antibiotic pre-treatment. Hence, the one study using antibiotic pre-treatment before FMT in the treatment of chronic pouchitis found no significant clinical improvement after treatment.<sup>182</sup> Moreover, antibiotic pre-treatment before FMT is still discussed in FMT for treatment of UC.<sup>144,145</sup>

The third limitation relates to the FMT treatment. In the FMT trials, stool handling was not performed under anaerobic conditions, which can influence the viability of anaerobic organisms.<sup>194</sup>

The fourth limitation concerns the analysis of the gut microbiota. The microbiome data included in the studies were generated from faecal samples, and the mucosal microbiota composition was not investigated as has been done in other studies.<sup>107</sup> Microbiome data were generated from 16S amplicon sequencing in Study I and III.<sup>124</sup> In 16S amplicon sequencing, the ability to correctly determine taxonomy using 16S amplicon data is debated, and connecting changes in microbiome composition to

specific taxa must be interpreted with caution.<sup>195,196</sup> Data on the microbial composition at species level was not available. Furthermore, changes of the gut microbiota found in pouchitis in general and a previous history of antibiotic treatments in particular will unavoidably influence the composition of gut microbiota in the patients and influence the microbial results.<sup>106,107</sup>

The fifth limitation concerns the control groups selected in Study I where we analysed the gut microbiota in different groups of patients and healthy controls. The composition of the gut microbiota of patients with chronic pouchitis was not compared with that of patients with UC or CD. It could be interesting to include patients with UC and compare the microbiota of these patients with patients with an inflamed IPAA, as performed by others.<sup>113</sup>

The sixth and final limitation concerns the systematic review of previously published studies on FMT in the treatment of chronic pouchitis. Quality assessment of the evidence in several of the included studies was graded as fair to poor quality. The majority of studies were small case and cohort studies with few patients, and only one study was a RCT. Furthermore, pronounced variances existed in the definition of disease activity and timepoint at treatment assessment. There was a great variation in preparation, delivery and number of FMT treatments in the included studies, hampering with the clarification of the overall efficacy and treatment approaches.<sup>162</sup>

#### 5.4. USE OF FMT AND WHERE TO MOVE FORWARD

Single-donor FMT is now an established and successful treatment for rCDI.<sup>150</sup> However, for other diseases, FMT may need to be optimised before achieving results as those seen in the treatment of rCDI.

Donor selection can potentially be a tool with which to improve the clinical efficacy of FMT. The term super-donor has been used to describe a faecal donor used for FMT with a high clinical efficacy in treated patients.<sup>137</sup> The term super-donor is, however, imprecise as the exact mechanism explaining why some donors are more suitable than others is unknown. The suitability of faecal donors in terms of high clinical efficacy in treated patients is often associated with a high engraftment of donor strains in patients.<sup>197</sup> Moreover, these donor strains are dominated by high microbial diversity and a high ratio of butyrate-producing bacteria such as *Prevotella*.<sup>197</sup> However, an RCT investigating anaerobic-prepared super-donor or autologous FMT in 66 patients with active UC found no difference in patients achieving steroid-free clinical remission (10% vs 13.9%).<sup>198</sup> Optimisation of FMT treatment using specific faecal donors is primarily investigated in patients with UC and has not been investigated in patients with chronic pouchitis. The anaerobic-prepared super-donors faeces were selected after excluding donors with *Bacteroides* enterotype, high abundances

of *Fusobacterium*, *Escherichia coli* and *Veillonella* and with the lowest microbial loads.<sup>198</sup> However, it is difficult to standardise FMT treatment as FMT recipients vary broadly in their engraftment of the donor microbiota, which we also illustrated. Besides selection of a suitable donor, safety needs to be in focus when including FMT as a treatment in the clinical setting. Safety of FMT is highly related to donor screening; thus, when following the current guidelines on donor screening and stool banking, FMT is assumed to be safe.<sup>133,141</sup> The COVID-19 pandemic highlighted the need for high-quality donor screening protocols to prevent transfer of pathogens to patients.<sup>199</sup> The faecal donor screening programme is dynamic, and has come to include more screening parameters over time, making selection of donors after screening more difficult in terms of achieving comprehensiveness. Another method with which to enhance or standardise FMT treatment may be using lyophilised FMT, which can concentrate the FMT product after removal of faecal sample water. In an RCT, Haifer et al. found that at week 8, eight (53%) of 15 UC patients in the FMT group achieved corticosteroid-free clinical remission with endoscopic remission or response compared with three (15%) of 20 patients treated with placebo (difference 38.3%; 95% CI 8.6–68.0;  $p=0.027$ ).<sup>155</sup> In general, comparison between FMT studies is difficult as several methods exist for stool processing, FMT delivery and comparison with FMT including autologous FMT. Furthermore, standard FMT treatment has yet to be defined.

The way to optimise the FMT treatment in patients with chronic pouchitis may not be selection of a donor with high engraftment of butyrate-producing bacteria, as described above, but instead selection of a “healthy” donor without a colon. In patients with an IPAA, the terminal ileum used to form the pouch will change over time after surgery to a more colon-like morphology, where villi are lost or blunted, and crypts develop.<sup>35</sup> Moreover, chronic inflammatory cell infiltrates are found in the lamina propria of the IPAA.<sup>36,37</sup> Even though the IPAA has a colon-like morphology, it is hard to assume that the environment in the IPAA is like that of the colon. This is also supported by the findings by us and others that the microbiota in the IPAA differs from the microbiota in a healthy colon.<sup>107</sup> Therefore, a patient with a normally functioning pouch may be a more suitable faecal donor for patients with chronic pouchitis.

Another way to investigate the mechanism of the FMT treatment is by looking into prediction of treatment failure or treatment success after FMT. In a meta-analysis by Azizullah et al. including 20 studies with 4,327 patients with *Clostridioides difficile* infection treated with FMT, several risk factors were associated with failure of FMT.<sup>200</sup> Risk factors included advanced age, severe CDI, IBD, use of non-CDI antibiotics before FMT, prior CDI-related hospitalisations, inpatient status and poor quality of bowel preparation.<sup>200</sup> In our subgroup analysis, we found no factors associated with failure of FMT; not even in patients who relapsed and who had a higher disease burden before treatment than patients in remission at follow-up. Donor- and patient-predictive biomarkers of response to FMT in patients with UC have also

been investigated in a systematic review including 25 studies.<sup>201</sup> Baseline clinical predictors of response were younger age, less severe disease and shorter disease duration.<sup>201</sup> Higher faecal species richness and higher microbial profile similarity between the recipient and donor were baseline patient microbial predictors of response. Following FMT, alpha diversity increases in responders, and increased abundance of *Clostridiales* clusters and genus *Bacteroides* and SCFA production were markers of FMT success.<sup>201</sup> Two research groups have investigated in meta-analysis donor strain engraftment for prediction of efficacy of FMT for multiple diseases including rCDI and UC.<sup>202,203</sup> Schmidt et al. found that dominance of donor strains and/or new strains in the recipient after FMT was highly predictable for successful engraftment, which was mainly driven by community dissimilarity and abundance ratios between the donor and recipient.<sup>202</sup> Ianiro et al. showed that microbial abundance and overall prevalence of species in donor and recipient before FMT were important for successful engraftment.<sup>203</sup> Furthermore, there was a link between successful donor strain engraftment and clinical improvement in the recipients after FMT.<sup>203</sup> Increased donor strain engraftment was associated with delivery of FMT from multiple routes and antibiotic pre-treatment.<sup>203</sup>

## 5.5. STRATEGY FOR TREATMENT FAILURE IN POUCHITIS

Pouchitis seems to become a bigger issue in the future. A published Danish population-based study found a 15% absolute and a 38% relative increase in the incidence of pouchitis in the population of patients undergoing IPAA surgery between 1996 and 2018.<sup>16</sup> However, treatment of chronic pouchitis is challenging, and it is questionable if a really successful treatment for chronic pouchitis exists. For now, the treatment strategy for chronic pouchitis is antibiotics; but in case of treatment failure, no well-documented treatment option exists.<sup>62</sup> Therefore, finding new treatment strategies is urgently needed.

In acute pouchitis, standard antibiotic treatment with either ciprofloxacin or metronidazole is often effective and the need for alternative treatment seems limited.<sup>62</sup> Probiotics have been tested in patients with active pouchitis, where the very concentrated probiotic preparation (VSL#3, 900 billions/sachet lyophilized viable bacteria, De Simone Formulation) using 3,600 billion bacteria/day for four weeks induced remission in 70% of the treated patients with mildly active pouchitis, and the PDAI score was significantly reduced.<sup>204</sup> Evidence for the use of probiotics in the treatment of pouchitis has been doubted, mainly due to the missing common microbiota signature alteration induced by the treatment.<sup>107</sup> However, probiotics may deserve a more prominent role in the prophylaxis of pouchitis. Patients treated with the probiotic preparation (VSL#3, De Simone Formulation, 2 packets containing 450 billion bacteria of different 8 strains *Lactobacillus paracasei/plantarum/acidophilus/bulgaricus*, *Bifidobacterium longum/infantis/breve*

and *Streptococcus thermophilus* each/day) immediately after ileostomy closure and for up to one year had significantly fewer episodes of acute pouchitis than patients treated with placebo (10% vs 40%) within the first year after surgery.<sup>205</sup> Moreover, treatment with a single probiotic *Lactobacillus rhamnosus* GG also appeared to have some efficacy in delaying the first episode of pouchitis compared with patients not treated with a cumulative risk at 3 years of 7% vs. 29%.<sup>206</sup>

As traditional treatment with antibiotics for now can have limited benefit in the treatment of chronic pouchitis, other treatment strategies for pouchitis need to be considered. In the treatment of chronic pouchitis, several alternative treatment options may be possible in case of failure of antibiotics. These options include corticosteroids and biologics.<sup>64</sup> However, large-scale head-to-head comparisons are needed before validation of the efficacy of these treatments can be performed. In small cohort studies, treatment with either oral budesonide-controlled ileal release (9 mg/day), oral beclomethasone dipropionate (10 mg/day) or topical tacrolimus for eight weeks caused a significant reduction in the PDAI score and 75%, 80% and 30% of the patients achieved clinical remission, respectively; and treatments were tolerable.<sup>207–209</sup> Treatment with biologics is starting to show promising results in the treatment of patients with chronic pouchitis. In a meta-analysis including a total of 313 patients, the effect of anti-TNF therapy with infliximab or adalimumab was evaluated in chronic refractory pouchitis.<sup>210</sup> The study reported a long-term clinical remission rate of 0.37.<sup>210</sup> Especially vedolizumab seems to be promising. Results from the EARNEST study including 102 patients with chronic pouchitis found that the rate of sustained remission at week 14 and 34 was higher for the vedolizumab group than for the placebo group (31% vs 10% at 14 weeks).<sup>211</sup> In an American multicentre cohort study including 83 patients, clinical response was achieved in 71% of patients and clinical remission in 19% of patients with chronic pouchitis treated with vedolizumab.<sup>212</sup> Probiotics (VSL#3, De Simone Formulation) have been tested in maintenance of remission in patients with chronic antibiotic-responsive pouchitis who were in clinical remission after 1 month of therapy with ciprofloxacin combined with rifaximin or metronidazole.<sup>213</sup> For both treatment durations, the remission rate for patients treated with 4 packets/day of VSL#3 for either 9 or 12 months was 85% compared with respectively 0% and 6% in the placebo groups.<sup>213</sup> The use of FMT from healthy faecal donors in the treatment of chronic pouchitis seems not to be beneficial as the previous RCTs and our study found no difference in remission rate compared with both placebo and autologous FMT.<sup>179,189</sup>

Diet can influence the disease activity of pouchitis, and a range of dietary components can influence pouch function to varying degrees by modulating upper gastrointestinal transit, small bowel water content and structure and fermentative activity of the pouch microbiota.<sup>53</sup> The British Society of Gastroenterology consensus guidelines on the management of IBD in adults recommend that all patients with a pouch should have a varied diet to achieve energy and nutrient requirements, including dietary fibres, which can have the benefit of regulating stool frequency, but no specific diet is

recommended.<sup>214</sup> The influence of the diet on disease onset and symptoms has been investigated. The influence of a Mediterranean diet (a diet based on the traditional cuisines of Greece and Italy, primarily containing plant-based foods) on onset of pouchitis in patients with IPAA was investigated in a study with 153 patients with a pouch.<sup>215</sup> Patients without pouchitis at inclusion were followed up for 8 years. The study reported that higher adherence to a Mediterranean diet trended to be inversely associated with the onset of pouchitis (log rank 0.17).<sup>215</sup> A small retrospective study examined the influence of the FODMAP (fermentable oligo-, di-, and monosaccharides and polyols) diet on pouchitis in seven patients with either an ileal pouch or an ileorectal anastomosis.<sup>216</sup> The FODMAP diet excludes poorly absorbed food, which increases faecal output; the FODMAP diet includes high lactose dairy, gluten, wheat, rye, barley, beans, some fruits and vegetables and high fructose corn syrup.<sup>216</sup> Five (71%) patients following the diet had reduced symptom of pouchitis, while the two (29%) patients deviating from the diet had chronic pouchitis.<sup>216</sup> Larger well-designed studies are needed to investigate the influence of diet on disease onset and symptoms in patients with pouchitis, together with the role of diet in the treatment strategy for chronic pouchitis.

## 5.6. FUTURE RESEARCH IN THE GUT MICROBIOTA IN CHRONIC POUCHITIS

The influence of the gut microbiota on the pathogenesis and treatment of chronic pouchitis remains only partly understood, and future research in this field is needed.

Interest in the influence of the gut microbiota on the pathogenesis of IBD and pouchitis also exists. An example is the Danish Centre for Molecular Prediction of Inflammatory Bowel Disease (PREDICT),<sup>217</sup> which investigates the cause and prognosis of IBD using longitudinal nationwide register data including microbiome data. A large nationwide register combined with biologic data, such as gut microbiome data, is needed to further investigate the pathogenesis of IBD, including pouchitis. To help understand the aetiology of pouchitis, longitudinal studies are needed of the impact of different factors influencing the gut microbiota in IPAA. Furthermore, when investigating the influence of the gut microbiota in pouchitis, it is important to understand the role of the composition of bacteria, vira and fungi in the context of the function of the gut microbiome.<sup>218</sup>

Currently, two ongoing clinical trials are investigating FMT for treatment of chronic pouchitis. The first trial, a single-centre trial from McMaster University, Canada, investigating FMT versus placebo for induction of clinical remission in patients with active pouchitis, plans to include 34 participants with an estimated primary completion of data collection in April 2023 (NCT03545386).<sup>219</sup> The study includes patients with active pouchitis defined as a PDAI score  $\geq 7$  and a history of recurrent

pouchitis. The trial appears to have a study design similar to that of the previously published RCTs FMT trials on chronic pouchitis using a single FMT delivered through a colonoscope and with sterile saline placebo as comparison.<sup>220</sup> It is questionable if this study will find a clinical benefit of FMT compared with placebo. However, the second trial, a multicentre trial from France investigating prophylaxis of recurrent pouchitis after FMT compared with placebo in patients with UC with IPAA, plans to include 42 participants with an estimated primary completion of data collection in September 2024 (NCT03524352).<sup>221</sup> This trial differs from the other FMT studies by including patients in remission with a PDAI score <7 to investigate if FMT is more effective than placebo in maintaining remission.<sup>221</sup> The study is the first study of its kind. In general, it seems that traditional FMT treatment from healthy faecal donors has a limited effect on patients with chronic pouchitis. Therefore, future focus should be on optimisation of the FMT procedure including donor selection, engraftment of donor microbiota and identification of specific donor microbial profile that promote a sustained clinical response if FMT should have a role in the treatment of chronic pouchitis.<sup>222</sup> Moreover, the role of the immune response in relation to clinical efficacy of FMT needs to be investigated in patients with pouchitis. It seems that the clinical efficacy of FMT cannot be explained only by changes in the gut microbiota or engraftment of the donor microbiota after FMT. However, it is unknown if alteration of the mucosal immune system is associated with clinical improvement in the patients after FMT.



## CHAPTER 6. CONCLUSION

The influence of the gut microbiota on chronic pouchitis and the use of FMT as a new strategy for treatment of this condition have been tested previously in several studies. However, the exact role of the gut microbiota in the disease mechanism of pouchitis remains unclear. Furthermore, the use of FMT as a new treatment modality for patients with chronic pouchitis remains questionable.

This thesis summarises current knowledge concerning the gut microbiota and use of FMT for treatment of patients with chronic pouchitis. Even though several studies have shown promising results, most previous studies investigating FMT for treatment of chronic pouchitis lack quality to justify implementation of FMT for treatment of patients with chronic pouchitis in clinical practice.

The main conclusion from this thesis is that non-pooled multi-donor FMT has no effect in inducing clinical remission in patients with chronic pouchitis compared with placebo. We observed a clinical remission rate of 44% and 40% after non-pooled multi-donor FMT in the open-label pilot study and the RCT, respectively. However, in the RCT, no difference in clinical remission rate was observed between the treated FMT group and the un-treated placebo control group.

In the present thesis, we also compared the gut microbiota profile in patients with chronic pouchitis with that of patients with a non-inflamed IPAA and FAP and that of healthy individuals. Patients with chronic pouchitis had a different microbiota profile than patients with a normally functioning IPAA and healthy individuals. Where genus *Bacteroides* was mainly associated with healthy individuals, the family *Enterobacteriaceae*, particularly genus *Escherichia*, was associated with patients with chronic pouchitis. Also, patients with chronic pouchitis had lower microbial diversity and richness than patients with a normally functioning IPAA.

These conclusions suggest that further research should focus on donor selection and optimisation of the FMT treatment to determine if patients with chronic pouchitis may benefit from this treatment in the future.



## CHAPTER 7. PERSPECTIVES

To further understand the influence of the gut microbiota and changes after FMT in patients with chronic pouchitis, changes in the microbiota composition in the mucosal biopsies must be investigated. We will compare the composition of the microbiota in the mucosal samples with the microbiota composition in the faecal samples. Furthermore, patients' immune response needs to be considered. The mucosal microbiota and markers for the immune response in pouchitis (CD14, TLR 4 and Interferon-stimulated gene 15) will be analysed in biopsies from the terminal ileum and IPAA collected before and after treatment with FMT and compared with biopsies taken from patients treated with placebo.

To achieve a better understanding of the role of FMT in patients with chronic pouchitis, we will analyse the diet of patients and that of their faecal donors. This analysis is expected to show if specific dietary elements can influence the effect of FMT. Furthermore, quality of life questionnaire (Short Inflammatory Bowel Disease Questionnaire and SF-36) studies will be conducted to investigate the influence of FMT treatment on patients' quality of life. Finally, long-term follow-up on patients treated with FMT will be performed and the results compared with those of patients treated with placebo; this analysis will include clinical symptoms, adverse events and gut microbiota data.

Selection of faecal donors seems crucial to optimise the treatment effect of FMT. For now, the criteria for selection of faecal donors are confined to selecting faecal donors without diseases or pathogens whose faeces can be transferred to patients. In the future, we will investigate if immunological markers such as human leukocyte antigen typing and immunoglobulin A in faecal donors and patients affect the clinical efficacy of FMT.

A patient with a normally functioning IPAA could be a new type of FMT donor for patients with chronic pouchitis. We have designed a study, which to our knowledge is the first proof-of-concept study, using a patient with a normally functioning IPAA as a donor for FMT treatment in patients with chronic pouchitis who have not previously benefitted from FMT from a healthy faecal donor. However, an RCT will be needed to conclude on the efficacy of FMT from a patient with a normally functioning IPAA as a donor to patients with chronic pouchitis. Future clinical trials on patients with chronic pouchitis need to include enough patients to gain sufficient statistical power, and future international multicentre trials may be needed.

Finally, it could be relevant to investigate the combination of FMT with immunomodulatory drugs, such as the biologic drug vedolizumab, in chronic pouchitis. The interestingness of such a study lies in the presumed interplay between the immune system and the gut microbiota in patients with chronic pouchitis. Also,

the use of FMT as a prophylactic treatment for recurrent pouchitis, as currently investigated in the French study, is relevant in the future research of FMT and pouchitis.

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# ABBREVIATIONS

ASVs	Amplicon sequencing variants
BMI	Body Mass Index
CD	Morbus Crohn disease
CDI	<i>Clostridioides difficile</i> infection
cPDAI	Clinical Pouchitis Disease Activity Index
ePDAI	Endoscopic Pouchitis Disease Activity Index
FAP	Familial adenomatous polyposis
FMT	Faecal microbiota transplantation
FODMAP	Fermentable oligo-, di-, and monosaccharides and polyols
GALT	Gut-associated lymphoid tissue
HCs	Healthy controls
HIV	Human immunodeficiency virus
hPDAI	Histological Pouchitis Disease Activity Index
IBD	Inflammatory bowel disease
IgA	Immunoglobulin A
IL	Interleukin
INF- $\gamma$	Interferon gamma
IPAA	Ileal pouch anal anastomosis
IQR	Interquartile range
M cells	Microfold cells
mPDAI	Modified Pouchitis Disease Activity Index
NGS	Next-generation sequencing

NOD	Nucleotide-binding oligomerization domain
OR	Odds ratio
PCA	Principal component analysis
PCR	Polymerase chain reaction
PDAI	Pouchitis Disease Activity Index
rCDI	Recurrent <i>Clostridioides difficile</i> infection
RCT	Randomised controlled trial
RD	Risk difference
RDA	Redundancy analysis
RR	Relative risk
SCFAs	Short-chain fatty acids
SD	Standard deviation
STAT-1	Signal transducer and activator of transcription 1
STEC	Shiga toxin-producing <i>Escherichia coli</i>
Th cells	T helper cells
TLR	Toll-like receptor
TNF- $\alpha$	Tumour necrosis factor alpha
Tregs cells	Regulatory T cells
UC	Ulcerative colitis
WGS	Whole genome shotgun sequencing
16S rRNA	16S ribosomal RNA
95%CI	95% Confidence interval



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