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ORIGINAL ARTICLE

Clinical results and microbiota changes after faecal microbiota transplantation for chronic pouchitis: a pilot study

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ABSTRACT

Objectives: Research evidence suggests that chronic pouchitis is associated with intestinal dysbiosis. Faecal microbiota transplantation (FMT) has been proposed as a possible treatment. We performed a 6-month prospective, open-label, single-centre cohort pilot-study (NCT03538366) to investigate if FMT could improve clinical outcome and alter gut microbiota in patients with chronic pouchitis.

Materials and methods: Nine adult patients with chronic pouchitis were included and allocated to 14 days FMT by enemas from five faecal donors, with a 6-month follow-up. Pouchitis severity was assessed using pouchitis disease activity index (PDAI) before and after FMT. Changes in gut microbiota, and engraftment of donor's microbiota were assessed in faecal samples.

Results: All patients were treated with FMT for 14 continuous days. Overall, four of nine patients receiving FMT were in clinical remission at 30-day follow-up, and three patients remained in remission until 6-month follow-up. Clinical symptoms of pouchitis improved significantly between inclusion and 14-day follow-up ($p = .02$), but there was no improvement in PDAI between inclusion (mean 8.6) and 30-day follow-up (mean 5.2). Treatment with FMT caused a substantial shift in microbiota and increased microbial diversity in six patients, resembling that of the donors, with a high engraftment of specific donor microbiota.

Conclusions: Symptomatic benefit in FMT treatment was found for four of nine patients with chronic pouchitis with increased microbial diversity and high engraftment of donor's microbiota. A larger, randomised controlled study is required to fully evaluate the potential role of FMT in treating chronic pouchitis.

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Pouchitis; IBD; microbiota; dysbiosis; faecal microbiota transplantation; IPAA

Introduction

Pouchitis is a common long-term complication after restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) in the surgical treatment of ulcerative colitis (UC), occurring in up to 60% of patients [1,2]. Pouchitis is characterised by inflammation primarily located to the IPAA and may extend to the ileum, resulting in clinical symptoms such as increased bowel movements, abdominal cramps, bloody stools and fever [1,3,4].

The pathogenesis of pouchitis is unclear. Changes in the intestinal microbial environment are hypothesised to induce a dysbiosis of the gut microbiota, which is associated with pouchitis [5,6]. Studies have found differences in the microbiota composition between patients with pouchitis and those with a non-inflamed pouch [5,7].

Pouchitis can be classified as acute or chronic depending on the duration of symptoms, or as antibiotic-dependent or antibiotic-refractory, according to the need for, and effect of, antibiotics [3,8]. Pouchitis is usually treated with ciprofloxacin and/or metronidazole, which in case of chronic pouchitis often fails [4,9,10]. Treatment of chronic pouchitis is challenging with limited therapeutic options [1,10–13], which may lead to pouch failure and need for surgical removal of the ileal pouch [14].

Faecal microbiota transplantation (FMT) has emerged as an established treatment for recurrent *Clostridioides difficile* infection [15], and studies have shown that FMT potentially inverts intestinal microbial dysbiosis, re-establishing an almost normal intestinal microbial environment [16,17]. A number of clinical trials suggest FMT as a promising therapy in UC [18–21], whereas FMT for chronic pouchitis has mainly been limited to case series with both positive and negative clinical results and varied assessment of the gut microbiota [22–28]. Our study is the first Danish prospective, open-label, single-centre cohort pilot-study to assess the clinical, endoscopic and histologic impacts, changes in gut microbiota and

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Supplemental data for this article can be accessed here.

engraftment of donor's microbiota after repeated multi-donor FMT, in treatment of chronic pouchitis.

Materials and methods

Trial design

Patients were included in a 6-month prospective, open-label, single-centre cohort pilot-study. All patients were allocated to treatment with FMT delivered by enema from five faecal donors for 14 consecutive days. Patients were asked to keep a daily diary including clinical Pouchitis Disease Activity Index (cPDAI), stool frequency as well as record any adverse events during the 14-day treatment. At baseline and 30-day follow-up, patients underwent a pouchoscopy with collection of biopsies and faecal samples, and the complete Pouchitis Disease Activity Index (PDAI) was assessed [29]. Additionally, faecal samples and questions concerning cPDAI, stool frequency and adverse events were collected on a monthly basis until end of follow-up after a 6-month period. A cut-off score of seven points in the PDAI score at 30-day follow-up was used to distinguish between remission (PDAI <7) and relapse (PDAI ≥ 7).

The primary endpoint was significant reduction of the PDAI score at 30-day follow-up compared to baseline at inclusion or a PDAI <7. Secondary endpoints were significant reduction in endoscopic and histologic PDAI scores at 30-day follow-up compared to inclusion, and increase in microbial diversity and richness in patients' stool after FMT.

Participants

Adult patients with chronic pouchitis were recruited between May 2018 and October 2018 at the Department of Gastrointestinal Surgery, Aalborg University Hospital, Aalborg, Denmark. In this pilot-study, the aim was inclusion of ten patients. Chronic pouchitis was defined as ≥ 3 episodes of pouchitis based on clinical symptoms, endoscopic signs of inflammation and histologic inflammation of pouch biopsies within the last year [30].

Inclusion criteria were as follows:

- Patients ≥ 18 years with an IPAA (>1 year).
- Clinical PDAI score ≥ 3.
- Chronic pouchitis (≥ 3 episodes of pouchitis during the last year).
- Treatment with ciprofloxacin and/or metronidazole for pouchitis (≥ 1 treatment during the last year).

Exclusion criteria were as follows:

- Immunosuppression (HIV, long-term treatment with prednisolone, anti-TNF-alpha therapy, chemotherapy).
- Pregnancy, planned pregnancy or breastfeeding.
- Faecal sample positive for enteric bacterial pathogens: *Salmonella*, *Campylobacter*, *Yersinia*, *Shigella*, *Vibrio*, toxin-producing *C. difficile*, diarrhoea-genic *Escherichia coli* (including Shiga toxin-producing *E. coli* (STEC)).

Faecal donors

Five faecal donors participated in the study, recruited from the Blood Bank at Aalborg University Hospital, Aalborg, Denmark, according to donor criteria (Supplementary 1). All donors were healthy and not receiving any medication. All donors were screened according to international guidelines for FMT with a questionnaire, as well as blood and faecal tests before and after a period of faecal donations during one month [31]. See detailed faecal donor protocol in Supplementary 1.

FMT sample preparation

The faecal samples donated to FMT treatment were processed according to the international consensus conference article on stool banking [32]. Faecal samples were delivered to our facility within four hours from defecation. Twenty grams faeces were diluted in saline and filtered to a final volume of 100 ml in each enema bottle; see the detailed FMT laboratory protocol in Supplementary 2. Each enema bottle contained faeces from one donor only. All bottles were stored at minus 80 degree until use.

Intervention

Patients were treated with FMT by enema once daily for 14 consecutive days. Any ciprofloxacin and/or metronidazole were stopped seven days prior to FMT. The 14-day treatment consisted of 14 individual enema bottles, each from a single donor, but from five different faecal donors in total (range 2–3 enema bottles from each donor). Patients were instructed to infuse one entire enema bottle with 100 ml suspended faecal material into the pouch once daily, and 'holding it' in the pouch for a minimum of one hour while lying down on the left. The first FMT dose was administered under supervision by the treating physician (SJK) at the Department of Gastrointestinal Surgery, Aalborg University Hospital, where the study visits took place. Patients performed the remaining FMTs by themselves, at home following thorough instruction.

Microbiota assessment

Sample preparation

Faecal samples were collected from patients for microbiota analysis at inclusion, at 30-day follow-up, and subsequently once monthly until end of follow-up at 6 months. Likewise, a faecal sample was collected once at inclusion from all donors. All samples were stored at minus 80 degrees before further investigation. DNA was extracted from all samples using QIAamp PowerFecal DNA Kit (QIAGEN, Copenhagen, Denmark) according to the manufactures instructions. Bacterial microbiota profiling (the hypervariable V4-region of the 16S rRNA gene) was used to analyse patient and donor stool microbiota. Detailed methods of these procedures are described in Supplementary 3.

Data analysis

The Wilcoxon signed-rank test was used to compare groups across clinical and biological variables. A paired Wilcoxon signed-rank test was used when comparing pre- and post-FMT samples of the same patient at inclusion and 30-day follow-up, respectively. A $p<.05$ was considered statistically significant. Data analysis were performed in R v. 3.6.0 through Rstudio v. 1.1.383 (http://www.rstudio.com).

The raw sequencing data was summarised into amplicon sequencing variants (ASVs) using an in-house pipeline AmpProc v5.1 (http://www.github.com/eyashiro/AmpProc/), based primarily on the USEARCH v10.0.240 workflow [33]. The ASVs were assigned taxonomy using SILVA LTP vers. 132 as reference database [34] (https://www.arb-silva.de/). Details of software and settings used are described in Supplementary 3. Data analysis was performed in R using the packages ampvis2 [35], vegan [36], data.table [37], ggplot [38], and tidyr [39]. Community richness was calculated using an observed number of ASVs and diversity was calculated using the Shannon index. For richness and diversity estimates only, all samples were rarefied to lowest observed sequencing depth (16,245 reads). Beta diversity was examined using principal component analysis (PCA) on Hellinger transformed ASV abundances. Filtering of ASVs with low variance, defined as >50% of samples associated with one value, were performed prior to PCA. Sample similarity was calculated using the Sørensen–Dice coefficient [40]. Testing for differential abundance were performed using DESeq2, with Benjamini–Hochberg adjusted *p*-values [41].

Ethics

The study was performed adhering to the requirements of Good Clinical Practice and the Revised Declaration of Helsinki. The study was registered in www.clinicaltrials.gov (NCT03538366). All patients provided signed written informed consent to participate. Consent for participation could be withdrawn at any time during the study period. According to the Danish Health Authority, FMT is not considered as a pharmaceutical and therefore no authorization by the Danish Medicines Agency was required. This study was approved by the Regional Committee of North Jutland in Denmark (N-20180008).

Table 1. Baseline characteristics of patients ($n=9$).

Age *mean (SD)*	51.5 (13.9)
Weight kg *mean (SD)*	74.0 (16.7)
BMI kg/m^2 *mean (SD)*	25.3 (5.7)
Height m *mean (SD)*	171.1 (9.1)
Male *n (%)*	3 (33.3)
Age of the pouch years *mean (SD)*	17.6 (6.7)
Continues use of antibiotic *n (%)*	3 (33.3)
Anti-diarrhoea drugs *n (%)*	7 (77.8)

BMI: body mass index; SD: standard deviation.

Results

Patient population

Overall, ten patients with chronic pouchitis were asked to participate. One patient withdrew consent prior to FMT treatment, ending up with a final cohort of nine patient that completed the 14-day treatment with multi-donor FMT. Patient characteristics are shown in Table 1. Four patients relapsed (meantime to relapse 4.3 days (range 0–9)) after the 14-day treatment with FMT and received antibiotic therapy. Of the remaining five patients, two experienced relapse after 15 and 52 days, respectively.

Clinical outcomes

Four of the nine patients (44%) were in clinical remission at 30-day follow-up, and three patients (33%) remained in remission throughout the 6-month follow-up.

The cPDAI score improved significantly from inclusion to 14-day follow-up ($p=.02$), and persisted at the 30-day and 6-month follow-up ($p=.06$ and $p=.10$) (see Table 2). However, there was no significant improvement of the PDAI score between inclusion and 30-day follow-up ($p=.22$) (see Table 2).

Endoscopic evaluation showed no improvement by endoscopic PDAI score between inclusion and 30-day follow-up ($p=.78$) (see Table 2) (see Supplementary Figure S1), which also applied to the histologic PDAI score ($p=1.00$).

Bowel movement frequency decreased from inclusion to 14- and 30-day, and 6-month follow-up (see Table 2), and faecal calprotectin decreased from inclusion to 30-day follow-up ($p=.39$).

Adverse events

Seven of the nine patients (77.8%) experienced one or several adverse events when treated with FMT (see

Table 2. Clinical outcomes before and after faecal microbiota transplantation.

	Inclusion	14-day follow-up	30-day follow-up	6-month follow-up
PDAI score *mean (SD)*	8.6 (3.4)	–	5.2 (4.5)	–
cPDAI score *mean (SD)*	3.7 (0.7)	1.6 (1.7)	2.0 (1.7)	0.7 (0.6)
PDAI endoscopic score *mean (SD)*	3.2 (2.0)	–	2.2 (1.8)	–
PDAI histologic score *mean (SD)*	1.7 (1.4)	–	1.0 (1.2)	–
Daily stool frequency *mean (SD)*	11.2 (4.9)	10.1 (2.9)	10.4 (3.2)	9.7 (3.5)
Faecal calprotectin µg/g *mean (SD)*	732.1 (1019.1)	–	152 (235.9)	–
Blood C-reactive protein mg/L *mean (SD)*	2.9 (2.2)	–	8.5 (11.9)	–

cPDAI: clinical pouchitis disease activity index; PDAI: pouchitis disease activity index; SD: standard deviation.

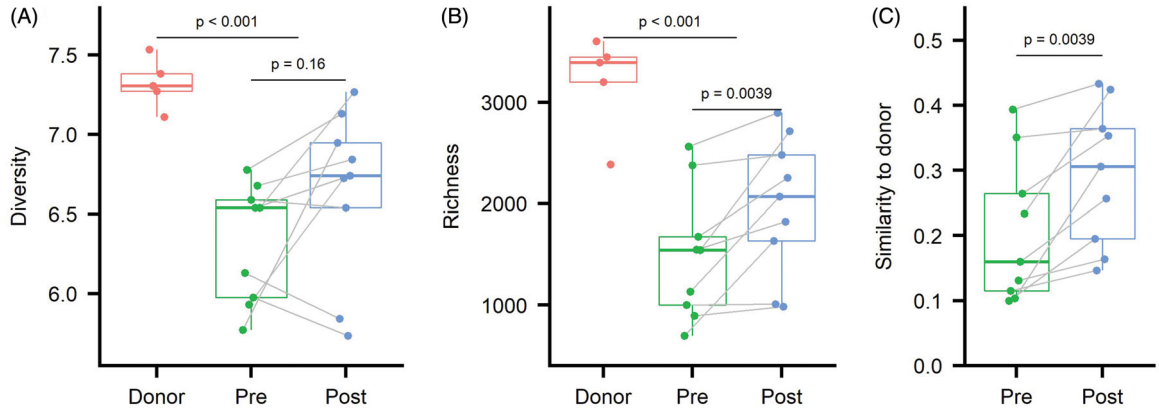

Figure 1. Microbial community characteristics in donors and chronic pouchitis patients. The Shannon diversity index (A) and number of amplicon sequencing variants (ASVs) for species richness (B) for donors and patients at inclusion and 30-day follow-up after faecal microbiota transplantation (FMT) for patients. (C) The similarity to donors for pre- and post-FMT patients samples, calculated as the Sørensen coefficient, comparing all ASVs present in the patient sample against the union set of all ASVs present in donors. Grey lines indicates pre- and post-FMT samples from the same patient. *p*-Values were calculated using a paired Wilcoxon rank-sum test.

Supplementary Table S1). All adverse events were assessed as minor, predominantly being abdominal pain. No serious adverse events including death or hospitalisation were observed during the treatment or follow-up period. Adverse events were only reported during the 14-day FMT treatment, none occurred during the follow-up period.

Microbiota analysis

Sequencing was successful for all samples (16,245–28,876 reads) and yielded sufficient reads to cover the community for both chronic pouchitis patients and donors determined by rarefaction analysis. Sequencing data are deposited in the Sequencing Read Archive (https://www.ncbi.nlm.nih.gov/sra/, accession number: PRJNA612771).Faecal samples collected from the patients had overall lower microbial diversity ($p < .001$) and richness ($p < .001$) compared to the donor faeces. An increased richness ($p = .004$) and marginally increased diversity ($p = .16$) were observed from inclusion to after FMT at 30-day follow-up (Figure 1(A,B)). Likewise, a higher similarity to the donors was observed after FMT ($p = .004$; Figure 1(C)), which were retained in two of three patients completing the 6-month follow-up (see Supplementary Figure S2).

Assessing relative abundance, members of *Ruminococcus* and *Bacteroides* genera were more prevalent in donor material than in patient samples (Figure 2(A,B)). The composition of the microbiota in the group of pouchitis patients was highly heterogenic, with some post-FMT samples enriched for members of the *Bacteroides* genera (Figure 2(A)). Through PCA it was possible to separate donor and patient samples (permutation test $p = .02$; Figure 2(C)). It was not possible to separate patients into pre- and post-FMT groups, because a subset of five patients shifted towards the donor communities (right-to-left of plot) whereas three diverged from the donors (left-to-right of plot; Figure 2(C)), and one patient did not shift. The bacterial taxa that contributed to shift in microbial composition towards the donor, were almost exclusively the genus *Bacteriodes*.

At the ASV level, we observed specific patterns of bacterial engraftment after FMT (Figure 3(A)). An overall high engraftment of ASVs unique to the donors' microbiota were seen for all patients (mean = 50.4%; SD = 12.3%). Patients were divided into two groups based on whether patients experienced relapse before/at 30-day follow-up (relapse groups) or were in remission (remission group). Comparing the percentages of community composition between patients in remission and relapsed (Figure 3(A)), more donor ASVs engrafted ($p = .016$), and less AVSs were shared between the donors and patients ($p = .016$). Engraftment of individual donors' microbiota, defined for each unique donor–patient combination as the percent of post-FMT ASVs unique to donor, revealed substantial patient and donor variability (Figure 3(B)). A two-way ANOVA of donor and patient effect on engraftment revealed the success of engraftment of donor's microbiota to be both patient-specific ($p < .001$) and borderline donor-specific ($p = .09$), with some donors (primarily no. 2) having better success at colonizing the patient than others after adjusting for patient effects (Figure 3(C)).

Microbial richness and donor-similarity increased in both relapse and remission groups, although not statistically significant (Figure 4(B,C)). Interestingly, pre-FMT samples of patients that relapsed before/at 30-day follow-up were on average characterised by less richness, as well as by better improvement of post-FMT samples, compared to the remission group (Figure 4(B,C)). Finally, patients in remission at follow-up had a more resilient microbiota, as measured by a higher community similarity pre- to post-FMT compared to patients experiencing relapse ($p = .016$; Figure 4(D)).

Discussion

This is the first Danish prospective, open-label, single-centre cohort pilot-study, which tested treatment with repeated multi-donor FMT in nine patients with chronic pouchitis and characterised their disease severity before and after FMT. Previously, FMT has been reported to reduce pouchitis symptoms in a limited number of small case and cohort studies [22,25,28]. Our study is the first open-label cohort study to evaluate clinical efficacy and the impact on microbiota, after

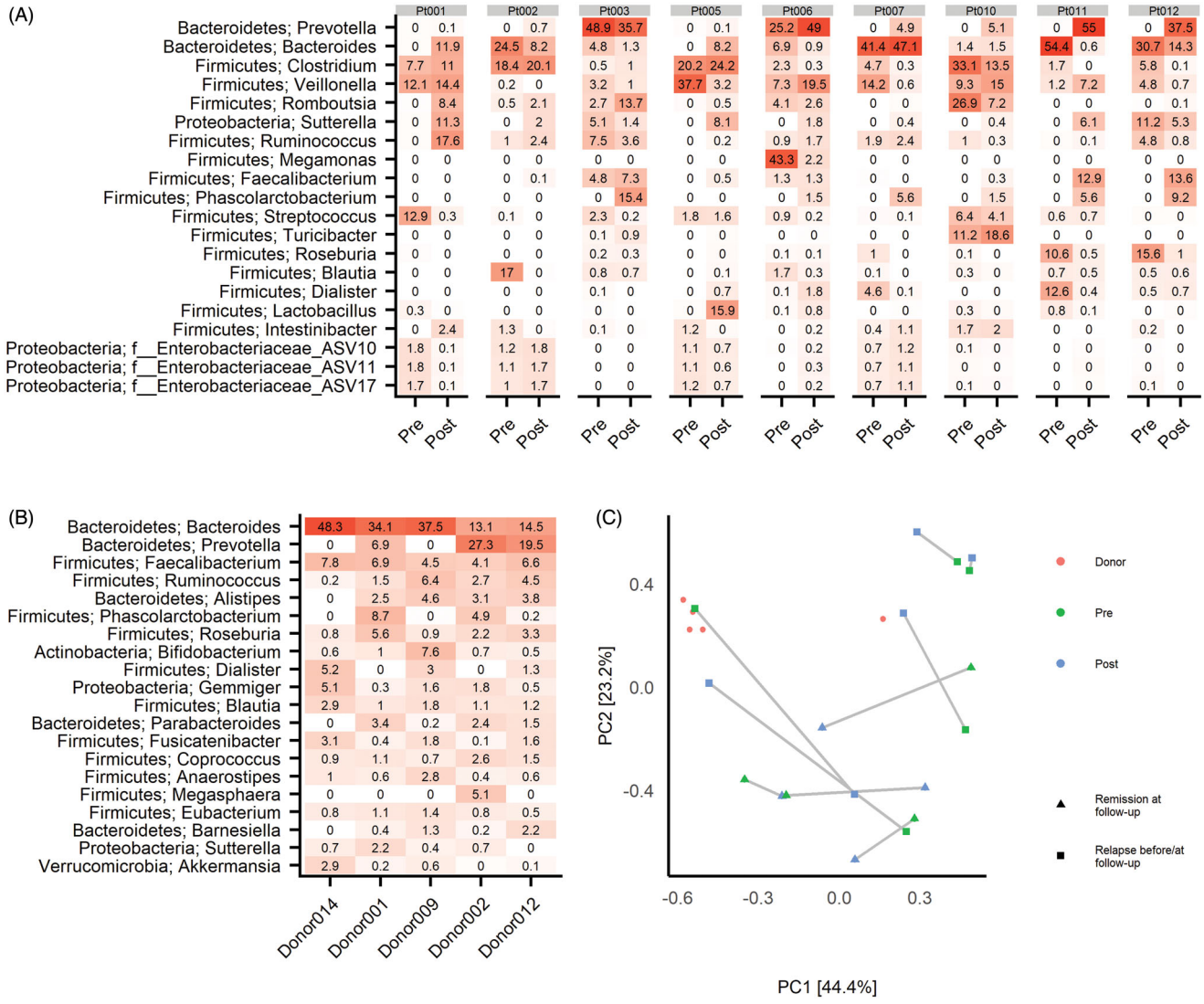

Figure 2. Microbial composition of patients with chronic pouchitis and healthy donors. The top 20 most abundant genera (relative abundance) with phylum names also provided, ordered from top to bottom by mean abundance are shown for patients with chronic pouchitis (A), and healthy donors (B). A principal component analysis (PCA) plot of the first two components for all patient and donor samples are shown in (C). Colours indicate donor, pre-FMT (inclusion before faecal microbiota transplantation (FMT)), and post-FMT (30-day follow-up after FMT) samples. Pre- and post-FMT samples from the same patient are connected by a grey line.

a 14-day treatment with FMT delivered by enema from several donors in patients with chronic pouchitis.

Overall, four out of nine patients were in clinical remission at 30-day follow-up after FMT. Although the clinical symptoms score was significantly improved at the 14-day follow-up, the disease severity of pouchitis, according to the PDAI score, did not indicate significant improvement after treatment with FMT. Likewise, no significant improvement in endoscopic or histological parameters were found after FMT.

The analysis of the microbiota indicated that patients with chronic pouchitis had significantly lower microbial diversity compared to the healthy donors, in accordance with previous findings in inflammatory bowel disease (IBD), including pouchitis [5,42]. The treatment with FMT by enema from several donors increased the microbial diversity with a switch towards the donors microbiota. In the patients microbiota, members of the *Bacteroides*, *Ruminococcus* and *Firmicutes* genera were less prevalent compared to healthy donor material, which is in accordance with previous findings [5,28]. Enrichment of the microbial composition after FMT was observed in the number of ASVs, despite lacking statistical power to detect a significant difference in the community diversity, which has been illustrated in other studies [25,28]. When dividing patients in two groups, patients in remission had a more resilient microbiota compared to patients experiencing relapse before/at 30-day follow-up, which could possibly predict relapse after FMT in these patients. Furthermore, the average microbial richness and diversity were lower in pre-FMT samples for relapsed patients compared to the pre-FMT samples from patients in remission. In accordance with pervious findings, the effect was systematic within the same patient for richness but not diversity, suggesting that presence/absence of certain taxa is important compared to their relative abundance [43]. The relative recovery of richness tended to be better in patients who experienced relapse, although this was not applicable for all.

We found a high engraftment of donor's microbiota compared to other studies [27,44], which may be explained by

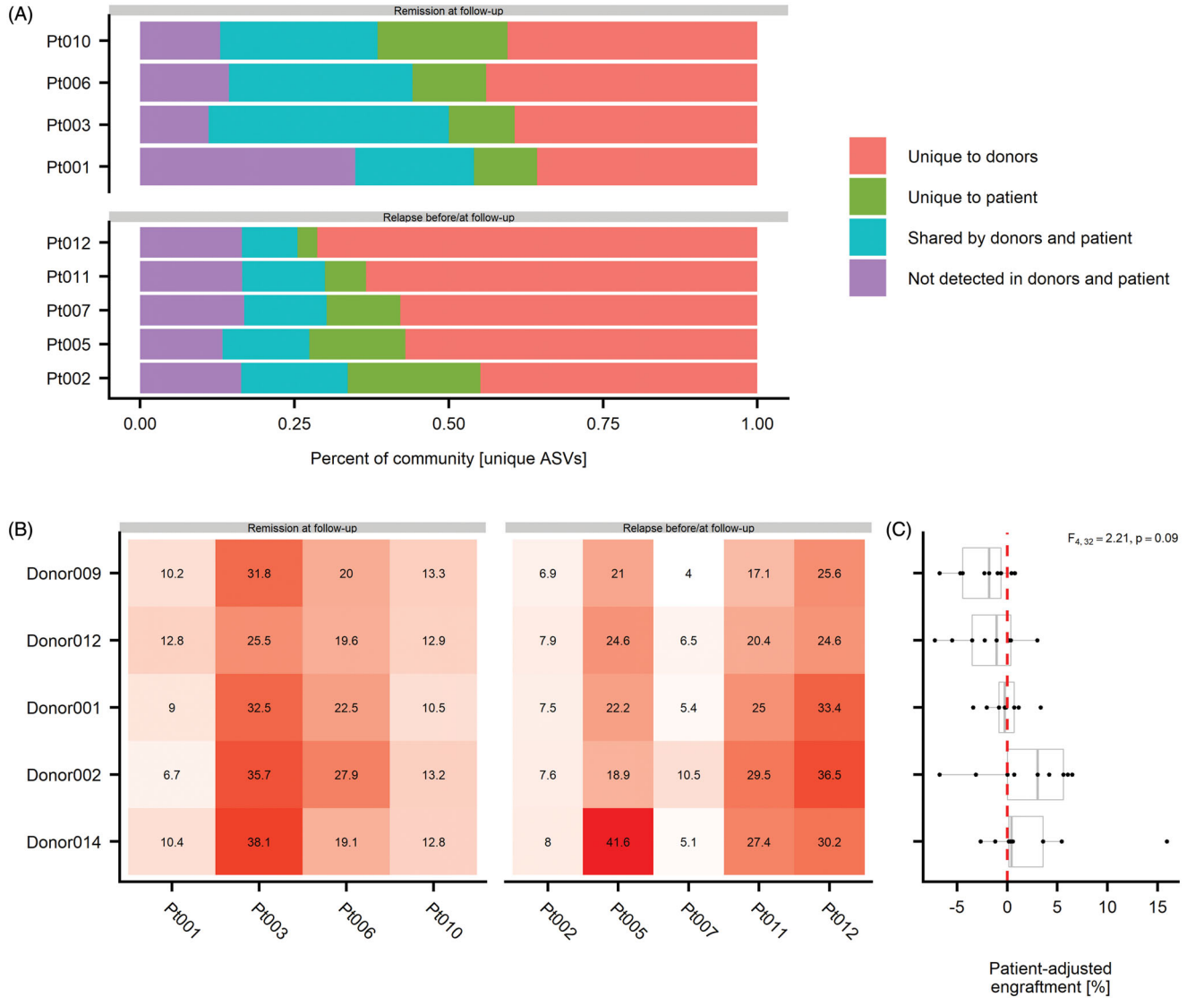

Figure 3. Engraftment of donor microbial community post-FMT (after faecal microbiota transplantation (FMT)) in patients with chronic pouchitis. (A) The percentage of post-FMT community that is unique to the donors, unique to the patient, present in both donors and patient, and undetected in pre-FMT sample, calculated as presence/absence of amplicon sequencing variants (ASVs). Patients are grouped by remission/relapse status at 30-day follow-up. (B) Cross-tabulation of the percent of ASVs in each patient on the x-axis that are also present in individual donors on the y-axis. In (**C**) the donor-specific engraftment of donor's microbiota are shown, by subtracting the column-mean (i.e. patient-specific mean) from data shown in (A). The *p*-value tests the donor-specific effect, calculated from a two-way ANOVA using donor and patient as variables.

the long-term FMT treatment. Generally, patients who experienced relapse seem to have a pronounced engraftment of donor's microbiota compared to patients in remission. This is also consistent with the decreased similarity post-FMT compared to the patients in remission, possibly indicating that these patients are more susceptible to donor-input, however, the exact meaning of this is unclear.

We included patients according to a clinical PDAI score $\geq$3, whereas other studies have only included patients with severe symptoms and endoscopic and histologic inflammation, with a high PDAI $\geq$9 [25,26]. In addition, the inclusion criteria with use of antibiotics was less strict, which meant that some patients were using antibiotics up to seven days before FMT, which might explain the low diversity in these patients. Stallmach et al. [25] included five patients with chronic antibiotic-refractory pouchitis with major pouchitis symptoms (PDAI $\geq$9), where four patients showed clinical remission after 1-7 FMT infusions, which was sustained in three patients after three months follow-up. This could indicate that FMT to chronic pouchitis requires several FMT treatments to achieve clinical remission similar to UC [18–21], whereas usually one FMT is sufficient to treat *Clostridioides difficile* infection [15]. The need for pre-treatment with antibiotics may be clinically relevant, as recommended to treat patients with recurrent *Clostridioides difficile* infection with vancomycin or fidaxomicin before FMT [31]. Selvig et al. [28] used pre-treatment with rifaximin in eight patient, which largely decreased the frequency of bowel movements and abdominal pain after FMT compared to those eleven patients who did not receive pre-treatment with antibiotics. We used a multi-donor approach to FMT by enema, whereas others have used single-donor FMT, which could influence the clinical response [23,25,26,28]. Multi-donor FMT is not recommended to the treatment of recurrent *Clostridioides difficile*

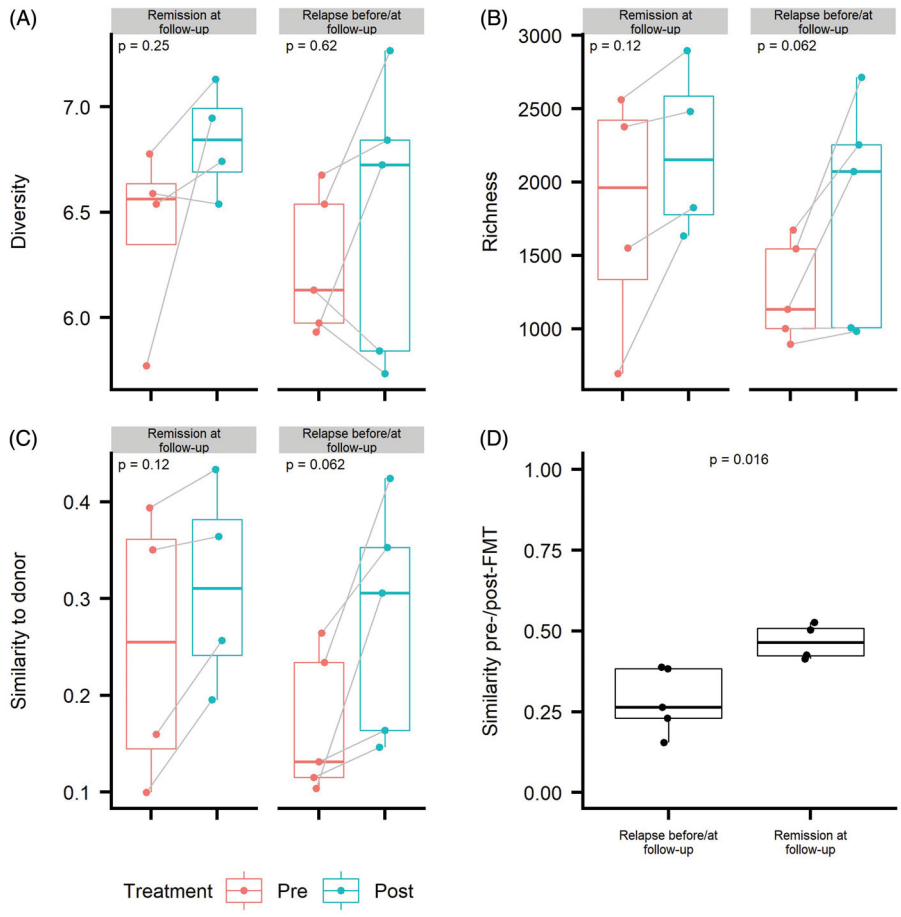

Figure 4. Community characteristics in patients, stratified by relapse before/at the 30-day follow-up or in remission. The Shannon diversity index (A) and number of amplicon sequencing variants (ASVs) for species richness (B) for patients pre- and post-FMT (before and after faecal microbiota transplantation (FMT)), split into relapse or remission. (C) The similarity to donors for pre- and post-FMT patients split by relapse, calculated as the Sørensen coefficient, comparing all ASVs present in the patient sample against the union set of all ASVs present in donors. (D) The similarity of patient post-FMT samples to the corresponding pre-FMT sample of the same patient, calculated using the Sørensen coefficient. *p*-Values for (A–C) were calculated using a paired Wilcoxon rank-sum test, while an unpaired test were done for (D).

infection based on reasons including safety and traceability. However, in the treatment of chronic pouchitis where single-donor FMT may have limited clinical effects, multi-donor FMT could be beneficial. Furthermore, the use of five donors to each patient were based on the thesis to increase microbial diversity, which has been proposed to be beneficial in the treatment of IBD [19,45].

Previous FMT studies for pouchitis have mainly administered donor-faeces into the jejunum by upper gastrointestinal tract endoscopy or into the pouch by lower gastrointestinal tract endoscopy [22–26]. Herfarth et al. [27] reported administration of FMT by capsules with no clinical effect in any of the four treated patients. Moreover, in the study by Stallmach et al. [25] patients received FMT intermittently, according to their symptom during a period of 3–4 weeks. A maintenance treatment with FMT could be more effective than one long-term treatment, as applied in our study.

We found a donor-specific effect of engraftment of donor's microbiota indicating that some donor microbiota engrafts better than others, however, the precise microbial compositions able to engraft needs to be further evaluated. This could indicate a need for precision FMT, and individualised microbiota screening of donors and patients prior to FMT, in order to get the best match [27].

In general, different treatment approaches for FMT need to be explored, including administration route, length of treatment, use of maintenance treatment, use of single- versus multi-donor approach, and pre-treatment with antibiotics. Primarily, a larger randomised controlled study is required to examine the true potential role of FMT in the treatment of chronic pouchitis.

The strength of our pilot-study is that all nine patients completed the 14-day multi-donor FMT treatment, with bacterial microbiota profiling before and after treatment. No patients experienced serious adverse events, and the minor adverse events as abdominal pain may even be related to the underlying inflammation of the pouch. For this reason, we find the procedure feasible and safe to use for patients with chronic pouchitis.

There are several limitations to this study. The small sample size does not give enough power to make any conclusion on the clinical effect of FMT for this group of patients, and the results should be interpreted with caution. However, one overall aim was to evaluate the safety and use of 14-day multi-donor FMT to chronic pouchitis patients before any implementation in a large scale. Moreover, four patients relapsed and withdrew from the study before the 30-day follow-up. As mentioned earlier, one FMT is sufficient to treat

Clostridioides difficile infection, however to UC and chronic pouchitis several FMTs seem to needed to achieve clinical remission. Therefore, a 14-day FMT protocol was used in our study, but based on our results, the length of FMT should be considered. A longer treatment length of FMT might cause a greater change in the microbiota with increased microbiota diversity, which could increase the clinical remission rate. However, a longer treatment length may influence the patients compliance and safety of FMT. Both patients with minor and major pouchitis symptoms were included, as a clinical PDAI score $\geq$3 was used as inclusion criteria. A larger study, including patients with major symptoms only, could be important to clearly assess the effect of FMT in chronic pouchitis. However, as chronic pouchitis is a rare disease in Denmark, further restriction of patients to include will make this study difficult to perform. Any antibiotics were stopped at baseline for all patients, however, treatment with antibiotics between patients differed. This should be corrected in future studies to achieve a more homogeneous group of patients for better comparison after FMT. Our microbiome data were generated on stools. Previous studies with FMT to chronic pouchitis have mainly also focused on faecal luminal microbiota, however assessment of the mucosal microbiota in pouchitis patients has been described [5]. Data on the mucosal microbiota could have been an interesting supplement to the stools. Future studies should investigate the changes in mucosal microbiota after FMT in chronic pouchitis patients, as mucosal microbiota may be more important in assessing IBD pathogenesis [46]. Donor faecal samples were analysed separately, since they were given as individual treatments. However, this could influence the results on engraftment of donor's microbiota, which therefore, also should be interpreted with caution. Finally, our microbiome data was generated from 16S amplicon sequencing [47]. We summarised data to ASVs to avoid clustering by nucleotide identity to maximize taxonomic resolution and reduce clustering biases [48]. In general, ability to correctly assign taxonomy using 16S amplicon data is questionable, caution should be taken when linking changes in microbiome composition to specific taxa [49,50].

In conclusion, we found that multi-donor FMT was safe and provided symptomatic benefit in four out of nine patients with chronic pouchitis, with an increased microbial diversity after FMT and high engraftment of donor's microbiota. A larger, randomised controlled study is required to examine the potential role of FMT in treatment of chronic pouchitis.

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Disclosure statement

Mads Albertsen is co-founder and owns part of the DNA analysis company DNASenseApS. Thomas Yssing Michaelsen and Jakob Brandt are part-time employed in DNASenseApS. No competing interests or conflict of interest for the remaining authors have been identified.

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