Title: Inflammatory Bowel Disease Outcomes Following Fecal Microbiota Transplantation for Recurrent *C. difficile* Infection

Short Title: IBD Outcomes after FMT

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Grant Support: The trial was funded by the Crohn's and Colitis Foundation. In addition, this work was supported in part by the Harvard Digestive Disease Center, NIH Grant P30 DK034854. Metabonomics studies were performed at the MRC-NIHR National Phenome Centre at Imperial College London; they receive financial support from the Medical Research Council (MRC) and National Institute of Health Research (NIHR) [grant number MC_PC_12025]. BHM is the recipient of a MRC Clinical Research Training Fellowship (grant reference: MR/R000875/1) and NIHR Academic Clinical Lectureship. The Division of Digestive Diseases and MRC-NIHR National Phenome Centre at Imperial College London receive financial and infrastructure support from the NIHR Imperial Biomedical Research Centre (BRC) based at Imperial College Healthcare NHS Trust and Imperial College London.

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Disclosures: JRA consults for and has research support from Finch Therapeutics Group. JRA, MF, CRK have research support from Finch Therapeutics and serves as clinical advisors to OpenBiome. YG, MS and ZK are employees/shareholders of Finch Therapeutics. ZK is an unpaid advisor to OpenBiome. BHM consults for Finch Therapeutics.

Author Contributions: JRA initiated study concept and design, acquisition of data, analysis and interpretation of data, and drafting of the manuscript. MF participated in developing study concept, study design, data acquisition and critical revision of the manuscript. YG, MS analyzed and interpreted microbiome data. BHM, AP, JAKM, JM, GFB, JLA, KIG and JRM generated, analyzed and interpreted the metabolomics data. MC, JM and JH, EP, SN participated in data acquisition. ZK participated in developing study concept, study design, interpretation of data and critical revision of the manuscript. AG, SVS, MB and CRK participated in data acquisition, interpretation of data and critical revision of the manuscript.

Summary: FMT has emerged as a CDI therapy however concerns regarding worsening of IBD activity post FMT exist. Secondary analysis from the first prospective FMT trial among IBD-CDI patients, revealing IBD outcomes are better than previously reported in retrospective studies.

Abstract:
Background: Recurrent *C. difficile* infection (CDI) is a challenge among patients with inflammatory bowel disease (IBD). Fecal microbiota transplantation (FMT) has emerged as a recurrent CDI therapy. Anecdotal concerns exist regarding worsening of IBD activity; however, prospective data among IBD patients is limited.

Methods: Secondary analysis from an open-label, prospective multi-center cohort study among IBD patients with 2 or more CDI episodes. Participants underwent a single FMT by colonoscopy (250mL, healthy universal donor). Secondary IBD-related outcomes included rate of *de novo* IBD flares, worsening IBD, and IBD improvement – all based on Mayo or Harvey Bradshaw index (HBI) scores. Stool samples were collected for microbiome and targeted metabolomic profiling.

Results: Fifty patients enrolled in the study among which 15 had Crohn's disease (CD) (mean HBI =5.8 ±3.4) and 35 had ulcerative colitis (UC) (mean Partial Mayo Score = 4.2 ± 2.1). Overall, 49 patients received treatment. Among the CD cohort, 73.3% (11/15) had IBD improvement, and 4 (26.6%) had no disease activity change. Among the UC cohort, 62% (22/34) had IBD improvement, 29.4% (11/34) had no change and 4% (1/34) experienced a *de novo* flare. Alpha diversity significantly increased post-FMT, and UC patients became more similar to the donor than CD patients (p=0.04).

Conclusion: This prospective trial assessing FMT in IBD-CDI patients suggest IBD outcomes are better than reported in retrospective studies.

Keywords: Inflammatory Bowel Disease; ulcerative colitis; Crohn's disease; fecal microbiota transplantation; microbiome; butyrate; *Clostridioides difficile* infection

Background:

Over the last decade, there has been an increase in the incidence and severity of *Clostridioides difficile* infection (CDI) that has been attributed to more virulent and treatment refractory strains[1, 2]; and its impact has been especially pernicious on inflammatory bowel disease (IBD) patients.[3-5] The prevalence of CDI in the IBD population was noted to be 2.5 to 8-fold higher with a 10% lifetime chance of getting the infection.[6-8] Since 1998, CDI related IBD hospitalizations have doubled and in-patient hospital mortality rose significantly from 5.9% to 7.2%.[9] Further, the in-hospital death rate of IBD patients is nearly five times greater when complicated by CDI.[9] Following an initial course of anti-CDI therapy, the CDI recurrence rate is 4.5-fold higher, and the prevalence of toxigenic *C. difficile* carrier state is 8-fold greater in IBD patients compared to those without IBD. [9] *Clostridioides difficile* may induce an IBD flare, worsen disease severity and negatively impact the clinical course.[9] In a retrospective study, *C. difficile* positive ulcerative colitis (UC) patients were twice as likely to be hospitalized, eight-times as likely to be seen in the emergency room and had nearly two-fold the colectomy rate compared to *C. difficile* negative UC patients for up to a year following the index hospitalization.[10] Additionally, it has been noted that CDI-IBD patients tend to improve on anti-CDI therapy suggesting that prompt eradiation of CDI may prevent colectomy, at least in the short term.[10] Overall, the data suggest that CDI among IBD patients is an important clinical challenge.

The use of fecal microbiota transplantation (FMT) has changed the CDI treatment paradigm and is now a guideline recommended therapy for recurrent CDI after several randomized trials compared it to standard of care antibiotics as well as placebo.[11-16] However, these trials commonly exclude IBD patients. In a retrospective cohort study, Kelly and colleagues demonstrated an overall cure rate of 94% in immunosuppressed patients, including patients with IBD.[17] This study also reported that 14% of patients post-FMT developed an IBD exacerbation, although this was not defined.[17] More recently, Khoruts and colleagues reported that patients with IBD-CDI were more likely to fail FMT,[18] leading to further questions surrounding the safety and efficacy of FMT in IBD patients with concurrent CDI. While placebo-controlled clinical trials of FMT to treat UC have shown early promise [19], the unique population of IBD patients with concurrent CDI remains poorly understood. Additionally, retrospective data suggesting IBD worsening after FMT for CDI remains an important clinical concern and there is a paucity of prospective data. Accordingly, we conducted a prospective study examining the impact of FMT on patients with IBD and CDI. We have previously published the CDI outcomes, revealing an 8% FMT failure rate. Here we aim to present the secondary IBD outcomes post FMT.

Methods:

Study design

Secondary analysis of IBD outcomes from an open-label, prospective, single-arm, multicenter cohort study at 4 tertiary care centers (Brigham and Women's Hospital (BWH), Indiana University, Brown University, and Mount Sinai Hospital). The study protocol was approved by the institutional review board at the Brigham and Women's Hospital, Indiana University, Brown University and Mount Sinai Hospital and all patients provided written informed consent prior to

participation (NCT03106844). An independent data and safety monitoring committee evaluated the trail regularly. Additionally, FDA approval via investigational new drug application (IND 17379) was obtained. All authors had access to the study data and reviewed and approved the final manuscript.

Study population

Patients with a confirmed diagnosis of IBD (ulcerative colitis (UC) or Crohn's Disease (CD) with any colonic involvement) and 2 or more confirmed episodes of CDI within 12 months, with the most recent episode occurring within 3 months, were enrolled. Polymerase chain reaction (PCR) or glutamate dehydrogenase (GDH) with enzyme immunoassay (EIA) for toxin were permitted for the qualifying CDI episodes, as the standard lab testing at each site differed. Patients with a total or subtotal colectomy, isolated ileal or small bowel CD, those pregnant or breastfeeding, those treated with vancomycin or metronidazole for more than 60 days or those that had undergone a prior FMT within 12 months were excluded.

Baseline assessments

Patients were assessed prior to the FMT and the following data was collected: IBD disease history, baseline IBD clinical scores (Harvey Bradshaw Index (HBI) for Crohn's Disease and Partial Mayo Score for UC), concurrent IBD therapy, CDI history, as well as baseline fecal calprotectin. Endoscopic scores (Mayo Endoscopic Score and the simple endoscopic score (SES)-CD were obtained at the time of FMT.

Interventions and follow up:

FMT donor material was produced at a large stool bank based (OpenBiome, Cambridge, USA). on a previously described protocol [20]. Briefly, healthy candidate donors underwent a rigorous health evaluation to rule out infectious and microbiome-mediated diseases. Subsequently, potential donors underwent a battery of stool and serological tests, aimed at screening for infectious diseases [21, 22]. Material was homogenized and filtered with a cryoprotectant and formulated into a 250mL preparation under GMP principles. FMT stored frozen at -80'C until shipped to sites. Material was thawed according to best practices prior to administration.

All patients received a single FMT via colonoscopy after undergoing a standard-of-care bowel prep with polyethylene glycol on the day before the colonoscopy. Anti-CDI antibiotics were held for 48 hours prior to the FMT. Colonoscopy was performed to the cecum with fecal material administered in the right colon. Each subject received material (250 mL) sourced from only one donor. Four unique donors were used across the study, and two of these supplied material for the majority of the patients.

Follow up assessments were performed 1, 8 and 12 weeks post-FMT. Clinical, laboratory assessments and stool testing for fecal calprotectin. All stool testing was performed centrally at the BWH clinical lab. A final safety assessment was performed at 6 months post-FMT.

IBD outcomes:
IBD outcomes assessed included changes in IBD activity in which we assessed for three outcomes utilizing clinical scores: 1) *de novo* IBD flare (defined as a Mayo or HBI score >4 at week 12 in the absence of CDI if Mayo or HBI score were 2 or less at baseline); 2) worsening IBD (defined as those with baseline active disease (Mayo or HBI score >4) and was defined as an increase in either HBI or Mayo score by 2 or more at week 12); 3) IBD improvement (defined as a decrease in Mayo or HBI score by 2 or more at week 12 compared to baseline without the need for rescue prednisone). IBD medication changes, if made based on ongoing or worsening disease activity were made by the referring IBD physician, not the study team, and were documented. Safety was also assessed as the proportions of adverse events through 6 months.

Assessment and analysis of the microbiome

Stool samples were collected for sequencing from donors and patients at baseline, 1, 8, and 12 weeks post-FMT. Samples were stored by flash freezing at -80^{o}C. DNA extraction, PCR amplification of the 16S rRNA gene's V4 region, and Illumina paired-end sequencing was performed at the University of Michigan core facility, as described previously [23].

Primers were trimmed, paired ends merged, and sequences were mapped to the SILVA database[24] forming operational taxonomic units (OTUs) clustered at 99% sequence identity, with a custom pipeline. Samples were sequenced to a median depth of 38,445 reads per sample, and 13,222 unique OTUs were identified across the study.

Samples were rarefied to the lowest sample read count (2,299 reads) for alpha diversity calculations or left unrarefied for other analyses. Paired t-tests were used to compare diversity or donor similarity before vs. after FMT, and independent t-tests were used for all other comparisons. Spearman rank was used for assessing correlation. Because previous reports have consistently shown low diversity in recurrent CDI microbiomes and that healthy-donor FMT increases diversity[25], one-sided tests were used for evaluating diversity changes post-FMT. One patient was discarded from analysis due to high pre-treatment donor similarity consistent with a sample labeling error.

Metabolomic assessment:

Ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) profiling and analysis of fecal bile acids was performed. The protocols used for fecal extract preparation[26] and analysis[27, 28] were as previously described. In addition, mass spectrometry data was analyzed using peakPantheR, an automated pipeline for the detection, integration and reporting of predefined features across a large number of mass spectrometry data files. Secondary bile acids were defined as those produced from primary bile acids via gut microbial 7-alpha-dehydroxylation, whilst tertiary bile acids were those produced from primary bile acids via processes involving other forms of microbial modification, e.g. 7alpha-/beta-isomerization of chenodeoxycholic acid (CDCA) to form UDCA.

Gas chromatography-mass spectrometry (GC-MS) for identification and quantification of fecal short chain fatty acids (SCFAs) was also performed. This was undertaken using previously-described protocols.[29] Samples analysis was performed on an Agilent 7890B GC system coupled to an Agilent 5977A mass selective detector (Agilent, Santa Clara, California). Analysis of data was performed using MassHunter software (Agilent).

Statistical Analysis

Categorical data were described using descriptive statistics (proportions and percentages). Continuous data were described using means and standard deviations (normally distributed data) or using medians and interquartile range (non-parametric data). Appropriate comparative statistical tests were chosen based in the variable types (categorical, dichotomous, continuous) and distribution (parametric, non-parametric) and were used to describe significant differences between intervention and control groups. Where appropriate, point estimates and confidence intervals and p-value two tailed with a significance level of 0.05 were reported. If patients withdrew or are lost to follow up, they were not replaced, and their data from the last assessment was carried forward.

Results:

50 participants were enrolled (between August 2017 and October 2019) among which 15 had Crohn's disease (CD) (mean HBI =5.8 ±3.4) and 35 had ulcerative colitis (UC) (mean Partial Mayo Score = 4.2 ± 2.1). Mean age of 43 years (range 21-91) and the cohort was primarily female (58%).A total of 49 patients received treatment, as one patient with UC withdrew prior to receiving FMT and was not replaced. Among the 49 treated, one patient was lost to follow-up after the week 1 visit, and week 1 data was carried forward. The primary outcome, FMT failure, has been previously reported.[30]

IBD outcomes

Patients had varying levels of IBD disease activity entering into the study. The mean baseline HBI score was 5.9 ± 3.5 with 20% between 1-3, 46.6% between 4-6, and 40% >6. The mean baseline partial Mayo score was 4.1± 2.1 with 48.5% between 1-3, 34.2% between 4-6, and 17% >6. The baseline IBD medications are listed in table 1. Among the CD cohort 26.7% were on prednisone at baseline, 60% were on a biologic, and 36.7% were on an immunomodulator. Among the UC cohort 48.6% were on prednisone at baseline, 51.4% were on a biologic, 48.6% were on mesalamine. IBD outcomes were calculated based on the baseline and week 12 clinical scores, or the last recorded score. If patients underwent a second FMT their scores following their second FMT were used. Based on the pre-specified definitions, in the CD cohort, 73.3% (11/15) of CD patient had IBD improvement, and 4 (26.6%) had no change in clinical scores. In the UC cohort, 62% (22/34) patients had IBD improvement, 29.4% (11/34) and 4% (1/34) experienced a *de novo* flare. This patient had a baseline partial Mayo score of 2 and was found to be 5 at the week 12 assessment.

In the CD cohort, among those noted to have improvement 54.5% (6/11) were able to taper off steroids by week 12. In addition, 3 (27%) of the improved patients were safely started on a biologic after FMT. All were noted to have active disease at baseline. In the UC cohort, 27.2%

(6/22) of those who improved were able to taper off steroids whereas 18% remained on a stable prednisone dose throughout. In addition, 3 patients were safely started on biologics post-FMT, all three had active disease at baseline.

Safety Outcomes
Overall the treatment was safe and well tolerated. Two serious adverse events (SAEs) were reported, neither were determined treatment related by the treating physician. The first was the above noted IBD flare. The subject was hospitalized for infliximab initiation and then was discharged. Another subject had worsening anemia that required a blood transfusion. The event was determined to be underlying disease related, not treatment related and resolved with a blood transfusion. There were several mild (grade 1 and grade 2) AEs reported, mostly gastrointestinal in a nature. Full list of AEs reported in Table 2.

Microbiome Outcomes
Alpha diversity significantly increased within one week of FMT in both patients with UC and CD (Figure 1A, $p<1e-17$), and this was sustained through week 12. Of the six patients who underwent a second FMT, alpha diversity did not further increase following the second treatment (Figure 1B). The microbial composition in UC patients became more similar to the donor than did CD patients ($p=0.040$), in particular among patients receiving one of the two major donors (Figure 2). Similarity to donor did not correlate with IBD clinical improvement.

The effect of FMT upon the fecal metabolome was also assessed. Post-FMT, fecal primary bile acids decreased, and secondary bile acids increased. Specifically, at each time point post-FMT, we observed a sustained, significant decrease in conjugated primary bile acids in stool (including taurocholic acid, the major endogenous trigger to *C. difficile* germination).[28] Conversely, the secondary bile acid, deoxycholate (which prevents the growth of *C. difficile*)[28] significantly increased at each time point post-FMT (Fig 3). Successful FMT was also associated with an overall restoration of short chain fatty acids (SCFAs) from initial very low levels to levels comparable to those found in the stool of healthy stool donors. Increases in butyrate was positively correlated with Firmicutes and negatively correlated with Proteobacteria in both IBD subtypes (Figure 4).

Discussion: Here we report secondary analysis and IBD outcomes from the first prospective trial to assess the effect of FMT in patients with IBD-CDI. We aimed to understand the magnitude of risk for potential IBD flare post-FMT in CDI-IBD patients.[17] We found that no patients with CD and only one patient with UC (4%) met the definition of *de novo* IBD flare. Moreover, the vast majority of patients had improved IBD clinical scores following elimination of CDI post-FMT. This finding contradicts previous reports. Our group performed a meta-analysis of studies assessing the rates of IBD flares or of a reported worsening clinical course, in which the pooled rate of IBD worsening was 14.9% (95% CI 10–21%).[31] When separated by patient population, the rate of worsening in IBD activity following FMT for CDI was 22.7% (95% CI: 13–36%) compared with FMT for IBD alone where the IBD worsening rate was 11.1% (95% CI 7–17%). This trial, in agreement with other randomized controlled trials of FMT for UC found only a marginal risk of worsening in IBD, at 4.6%, (95% CI: 1.8–11%) suggesting reporting bias in small retrospective studies.[31] The definition of flare or IBD worsening also varied between

previous retrospective studies and there were often no set definitions. To standardize this, we set three definitions: IBD worsening, IBD improvement and *de novo* flare, to account for baseline IBD activity. We found that many patients had active disease prior to FMT and continued to have active disease post-FMT. Given the prospective design of this trial it was clear that active disease post-FMT did not represent FMT treatment related flare. We did find that with swift eradication of CDI, patients with active disease at the time of FMT were able to safely start appropriate IBD therapy and ultimately lead to improvement in their disease activity.

The microbiome analysis revealed significant increase in alpha diversity as early as 1 week post-FMT and this increase was sustained. Interestingly, patients that received a second FMT, did not experience an increase in alpha diversity further, but did all achieve cure with a second FMT from the same donor. We had no patients who required more than 2 FMTs or additional anti-CDI therapy beyond that. We did find that the microbiomes of patients with UC became more similar to the donors than patients with CD, as well as had higher engraftment efficiency. This convergence may shed light on why investigators are seeing early success with FMT as a therapy for UC. We, however, did not observe a correlation between donor similarity and clinical improvement in this trial. We did appreciate patterns in restoration of bile acid profiles post-FMT similar to non-IBD patients as well as an increase in butyrate post-FMT correlated with an increase in Firmicutes, including *Faecalibacterium*, a major contributor to butyrate production in the healthy gut..[32] One potential inference of this observation is that restoration of gut microbiome functionality – as well as restoration of composition – is of key importance to the efficacy of FMT. In particular, we noticed early and maintained restoration of both bile acid and SCFA profiles to pre-morbid patterns after FMT. While already well-established that these metabolites are contributory to the pathogenesis of CDI and efficacy of FMT in its treatment, there is also growing evidence for their importance to IBD as well. For instance, secondary bile acids have recently been shown to be reduced in UC patients with pouches, and secondary bile acid supplementation is associated with reduced inflammation in animal models of colitis.[33, 34]

This study had several limitations. We used subjective clinical scores to assess IBD activity and may impact interpretation of disease activity in the context of an open-label study, especially with the overlap of symptoms between CDI and active IBD; however, placebo effect in this very ill patient population was likely to be negligible. Additionally, we acknowledge that referring physicians were able to change IBD therapy as they felt necessary; therefore, we were not able to comment on FMT as a treatment for IBD. We do feel that there are several strengths, however. We undertook the largest prospective trial to follow patients systematically post-FMT, and to assess for IBD clinical activity.

This study was able to demonstrate that FMT for the treatment of recurrent CDI in patients with IBD was safer and better tolerated than has been previously reported in retrospective studies. We did not appreciate IBD worsening and only one patient met the definition of a flare *de novo*, highlighting the fact that many of these patients have active disease prior to FMT and will continue to have active disease post FMT. Appropriate treatment with biologics post-FMT after eradication of CDI was safe and led to overall IBD improvement.

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Table 1: Inflammatory Bowel Disease Baseline Medications

Crohn's Disease	N=15
Steroids % (n)	
Prednisone	26.7% (4)
Budesonide	6.7% (1)
Biologics	
Vedolizumab	20% (3)
Certolizumab	13.3% (2)
Adalimumab	6.7% (1)
Infliximab	6.7% (1)
Ustekinumab	6.7% (1)
Cyclosporine	6.7% (1)
Mesalamine	
Oral 5-ASA	13.3% (2)
Rectal 5-ASA	6.7% (1)
Immunomodulators	
Azathioprine	13.3% (2)
Methotrexate	13.3% (2)
Ulcerative Colitis	N=35
Steroids	
Prednisone	48.6% (17)
Budesonide	5.7% (2)
Rectal Corticosteroids	8.6% (3)
Biologics	
Adalimumab	8.6% (3)
Vedolizumab	22.9% (8)
Infliximab	17.1% (6)
Tofacitanib	2.9% (1)
Cyclosporine	2.9% (1)
Mesalamine	
Rectal 5-ASA	11.4% (4)
Oral 5-ASA	57% (20)
Immunomodulator	
Methotrexate	2.9% (1)

Table 2: Adverse Events by Body Site

Body system	N=49 % (n)
Gastrointestinal	
Diarrhea	44.9% (22)
Rectal Bleeding	16.3% (8)
Abdominal Pain[+, ++]	26.5 (13)
Nausea	10.2% (5)
Vomiting	6.1% (3)
Heart Burn	4.1% (2)
Constipation	2% (1)
Rectal Abscess	2% (1)
Right lower Quadrant Pain	2% (1)
Gastritis	2% (1)
Bloating	2% (1)
Discoloured Stool	2% (1)
IBD flare*	2% (1)
Systemic	
Fever	10.2% (5)
Fatigue	6.1% (3)
Chills	4.1% (2)
Night Sweats	2% (1)
Anemia**	2% (1)
Burning pain on feet	2% (1)
Pedal bilateral edema	2% (1)
Respiratory	
Cough	2% (1)
Dermatologic	
Shingles	2% (1)
Eczema	2% (1)
Cyst	2% (1)
Swollen Lymph Nodes	2% (1)
Rash	2% (1)
Polymorphous Light Eruption	2% (1)
Skin Abscess	2% (1)
Neurological	
Migraine	4.1% (2)
Genitourinary	
Yeast Infection	4.1% (2)
Kidney Stones	2% (1)
Musculoskeletal	
Muscle Soreness	2% (1)
Back pain	2% (1)
Infection	
Oral Infection	2% (1)
Pharyngitis	2% (1)

*Grade 3 IBD flare that resulted in a hospitalization in one subject reported during the week 8 follow-up. Subject was treated with Remicade and discharged from hospital. **Subject with Anemia required blood transfusions and reported the event during their

week 8 follow-up. The event was deemed to be disease related not treatment related, resolved with transfusion. [+]Subject went to ER for general GI pain and reported the event during their week 26 follow-up. Subject was given Percocet to take as needed. [++]One subject visited the ER due to Abdominal pain and reported this event during their week 12 visit. Subject was treated with pain medication.

Figure 1: Alpha diversity after (A) first FMT and (B) in patients receiving second FMT. Increases were significant after first FMT for all patients pooled ($p<1e-17$) and when stratified by IBD subtype (UC $p<1e-13$, CD $p<1e-5$).

Figure 2: Similarity to the donor post-FMT measured by Jensen-Shannon Divergence, stratified by donor and IBD subtype. Data from two patients receiving other donors not shown. Change was measured as difference in similarity post-FMT vs baseline and was significantly different between UC and CD patients (all patients, $p=0.040$; Donor 1 and 2 only, $p=0.038$, two-sided t-test). Differences between the subtypes were largest among recipients of Donor 2.

Figure 3: Log fold-change in primary and secondary bile acids following FMT. For each patient, all values post-FMT were averaged, then divided by the patient's baseline. Significant changes are indicated (* indicates $p<0.05$, ** indicates $p<0.005$, two-sided t-test on log abundance).

Figure 4: Associations between changes in fecal butyrate and major phyla abundances. Changes were measured between baseline and week 12 for two major phyla: Firmicutes (left) and Proteobacteria (right). Spearman correlations were significant for Firmicutes across all patients ($p=0.021$) and when stratified by IBD subtype (UC, $p=0.037$; CD, $p=0.039$).