Antibiotic cocktail for pediatric acute severe colitis and the microbiome: the PRASCO randomized controlled trial

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Funding: This study was funded by a grant from the International Organization of IBD (IOIBD) and from the Helmsley Charitable Trust.

Conflict of interest:

KLK received consultation fees from Abbvie, Biocodex, Ferring, MSD, and Tillotts Pharma
HV, SC, BY, DM, EL have no conflict of interest to declare.
DT received last 3 years consultation fee, research grant, royalties, or honorarium from Janssen, Pfizer, Hospital for Sick Children, Ferring, Abbvie, Takeda, Biogen, Atlantic Health, Shire, Celgene, Lilly, Neopharm, Roche

AMG has received in the last 3 years consultation fee, research grant, or speaker honorarium from Abbvie, Celgene, Ferring, Gilead, Janssen, Lilly, Merck, Nestle, Pfizer, Roche, Shire.
AL has received consultation , IP, or DSMB fees, research grants, or speaker honorariums from Abbvie, Takeda, Jannsen, Nestle health science, Celgene, Ferring and Megapharm
MA received honorarium by Abbvie
AA received consultation and lectures fees from Abbvie and research grants from Abbvie and Janssen.

ABSTRACT

Background: Alterations in the microbiome has been postulated to drive inflammation in IBD. In this pilot randomized-controlled trial we evaluated the effectiveness of quadruple antibiotic cocktail in addition to intravenous-corticosteroids (IVCS) in acute severe colitis (ASC).

Design and procedures: Hospitalized children with ASC (PUCAI ≥65) were randomized into two arms: the first received antibiotics in addition to IVCS (amoxicillin, vancomycin, metronidazole, doxycycline/ciprofloxacin (IVCS+AB)), while the other received only IVCS for 14 days. The primary outcome was day-5 disease activity (PUCAI) score. Microbiome was analyzed using 16S rRNA gene and metagenome.

Results: 28 children were included: 16 in the AB+IVCS and 12 in the IVCS arms (mean age 13.9±4.1 years and 23 (82%) with extensive colitis). The mean day-5 PUCAI was 25±16.7 vs 40.4±20.4, respectively (p=0.037). Only 3 and 2 children, respectively, required colectomy during one-year follow-up (p=0.89). Microbiome data at time of admission were analyzed for 25 children of whom 17 (68%) had a predominant bacterial species (>33% abundance); response was not associated with the specific species while decreased microbiome diversity at admission was associated with day-5 response in the IVCS arm.

Conclusion: Patients with ASC have alterations in the microbiome characterized by loss of diversity and presence of predominant bacterial species. Quadruple therapy in addition to IVCS improved disease activity on day 5, but larger studies are needed to determine whether this is associated with improved long-term outcomes (clinicaltrials.gov NCT02033408).

INTRODUCTION

It is believed that the pathogenesis of inflammatory bowel diseases (IBD) is related to disequilibrium between an altered immune response and the enteric microbiome. We have previously shown in the prospective Outcome of Steroid therapy in Colitis Individuals (OSCI) study that children with acute severe colitis (ASC) responding to intravenous steroids have a more diverse microbiome than non-responders (1). The mucosa stands as a barrier between the microbiome and the host, and thus the increased permeability of the gut seen in IBD may be associated with translocation of micro-organisms, triggering chronic inflammation.

Antibiotic therapy has long been used in IBD with conflicting and varying results (2). In ASC, three clinical trials showed no benefit to *intravenous* antibiotics including metronidazole (3), ciprofloxacin (4), and metronidazole with tobramycin (5), all in combination with intravenous steroids. In contrast, three other placebo-controlled trials showed a significant benefit to *oral* poorly absorbed antibiotics in ASC, including oral tobramycin (6), oral vancomycin (7) and oral rifaximin (8). The effectiveness of oral vancomycin was further demonstrated by us and by others in case series, often when used primarily to treat primary sclerosing cholangitis (PSC) (9, 10).

In a series of studies, a Japanese group provided evidence that an anti- *Fusobacterium varium* oral antibiotic cocktail (14 day triple therapy with amoxicillin, metronidazole, and tetracycline) may be effective in ulcerative colitis (UC) (11) (12, 13) (14) (15). The cocktail showed encouraging results in two cohorts of adults with steroid-refractory and steroid-dependent UC (12, 13) and in a small randomized controlled trial (RCT) conducted among such patients (14). In another RCT of 210 adults with mild to severe UC, improvement at 3 months was shown in 45% of those receiving the antibiotic cocktail compared to 23% with

placebo; remission was superior in the antibiotics arm only at 1 year (15). Other microbes, such as *Ruminococcus gnavus* (16) and *E. coli* (17) are enriched in the gut of UC patients.

Even if *Fusobacterium* are not the causative organism in UC, it is tempting to speculate that at least some episodes of ASC are triggered by a yet-unknown enteric infection, similar to *Clostridium difficile* or cytomegalovirus that have been associated with refractory acute exacerbations. Based on that speculation and building on the studies with the Japanese cocktail and numerous studies on the effect of oral vancomycin, we trialed a quadruple therapy cocktail of oral metronidazole, amoxicillin, vancomycin and doxycycline (or ciprofloxacin in younger children; hereafter referred to as the Jerusalem cocktail) in children with ASC. We published our experience of the first treated 15 children, of whom almost half responded (18). Subsequently, Kordy et al reported 8 children with steroid-resistant or dependent-UC treated with the cocktail, of whom 7 (88%) responded and 3 (38%) entered remission (19). Further data concerning 63 children from Philadelphia treated with various versions of the Jerusalem and Japanese cocktails showed overall 40% remission rate at 3 weeks; 26 of the cohort were hospitalized with ASC of whom 7 (27%) entered remission at 3 weeks (20).

Since there are no controlled studies in children to evaluate the effectiveness of antibiotics in ASC, we aimed in this PRASCO trial (**P**ediatric **R**andomized trial of **A**ntibiotics in acute **S**evere **CO**litis) to evaluate the effectiveness of the Jerusalem antibiotic cocktail in pediatric ASC in a 1-year open label, investigator-blinded RCT. We also aimed to explore the microbiome pattern of children admitted for ASC, before and during treatment.

METHODS

This was an "add-on" open label randomized investigator-blinded multi-center international trial in children receiving intravenous steroids for ASC, comparing two arms: the first received antibiotics in addition to steroids ("AB+IVCS" group), while the other received only intravenous steroids ("IVCS" group) (clinicaltrials.gov NCT02033408). The study was approved by the local ethics board at each of the participating sites.

Eligibility criteria

Children aged 2-18 years with established diagnosis of UC using standard criteria (21, 22), were eligible if they had severe disease (i.e. pediatric UC activity index (PUCAI)≥65), required admission for IVCS and had negative stool analysis for culture, enteric parasites, viruses and *C. difficile* toxins (23). Although the protocol allowed including children with Crohn's colitis, none were eventually included. The protocol initially mandated failing at least 1-week of oral prednisone and being anti-TNF naive, but these criteria were later removed to facilitate recruitment, on condition that the anti-TNF dose has been stable for at least 8 weeks. Exclusion criteria were disease confined to the rectum, use of antibiotics during the last month, any proven infection, the presence of fever thought to be unrelated to the inflammatory process, and known allergy to at least two antibiotics from the cocktail. The necessity for initiating immediate treatment in the setup of ASC did not allow for a screening period and thus all eligible children were randomized while awaiting stool infectious screening; positive results led to immediate exclusion from the trial and the antibiotic treatment was discontinued.

Interventions (Table 1, Figure 1 and 2)

Patients were randomized into two groups:

1. The "IVCS" group received standard therapy with methylprednisolone only (1.5mg/kg up to 60mg daily in two divided doses)
2. The “AB+IVCS” group received the steroids as well as oral antibiotics for 3 weeks:
    a. Vancomycin 250mgX4/day (children <8 years of age- 125mgX4/day)
    b. Amoxicillin 50mg/kg/day divided into 3 doses (up to 500mgX3/day)
    c. Metronidazole 5mg/KgX3/day (up to 250mgX3/day)
    d. Doxycycline 2mg/kgX2/day (up to 100mgX2/day) (children <7 years of age ciprofloxacin 10mg/KgX2/day (up to 250mgX2/day)

Known allergy to one of the drugs led to substitution with oral gentamicin (2.5mg/KgX3/d). Dose was rounded to the closest possible whole pill dose. As recommended in ASC, 5-ASA was discontinued upon admission and food continued liberally as tolerated; total parenteral nutrition (TPN) was allowed as needed (23). Topical rectal treatment was allowed if administered without dose change in the week preceding admission.

Outcomes

The primary outcome was day-5 PUCAI score, which is used to decide upon second line therapy in pediatric ASC (23) and is highly correlated with endoscopic appearance of the colonic mucosa with a concordance of >80% (24-27). The European Medicines Agency (EMA) recommends using the PUCAI as the primary endpoint when endoscopic assessment is not available as in pediatric ASC where sigmoidoscopy is recommended only in non-responders (23). We supplemented the analysis by serial fecal calprotectin measures as a secondary outcome. Other secondary outcomes included remission rates (i.e. PUCAI<10),

need for second line therapy (anti TNF, cyclosporine, tacrolimus or colectomy), and rate of *C. difficile* infections after treatment.

Procedures

PUCAI score, standard blood results and explicit clinical details were recorded on days 0, 3, 5, 7, 10, 14, at discharge, 1 month, 2 months, 3 months, 6 months, and 12 months after randomization. Deterioration after 3 days (i.e. PUCAI increase by at least 20 points) or nonresponse after 5 days (i.e. PUCAI decrease by <20 points or absolute PUCAI ≥65) were defined as treatment failure. Once PUCAI<35 was achieved, patient discharge was allowed and corticosteroids given orally in tapering doses based on a standardized protocol (28). After 14 days new maintenance therapy with thiopurine/anti-TNF/5-ASA, based on the discretion of the treating physician, was allowed.

Randomization, concealment of allocation and blinding

Patients were randomized by sealed, opaque and numbered envelopes in blocks of four at a 1:1 ratio. This was not a placebo-controlled study but the investigator scoring the PUCAI at each visit and the lab technicians performing the calprotectin and microbiome analyses were blinded to the allocation. The clinicians and patients were instructed not to discuss the allocation with the investigator.

Samples

Stool was collected for fecal calprotectin, *C. difficile* and microbiome analyses at days 0, 5, 7, discharge, 14, and 30, as well as at 2, 3, 6 and 12 months following randomization. Stool samples were frozen at -80°C within 4 hours from collection and if that was not possible, another sample was collected with 5ml of ethanol as fixative and processed within 30 hours

(centrifuged 10min at maximal speed, then supernatant (ethanol) was discarded). Samples were shipped on dry ice to Shaare Zedek Medical Center for calprotectin analysis (performed by IDK Calprotectin ELISA kit from Immundiagnostik) and to the Broad Institute's Microbial 'Omics Core for microbiome analyses.

DNA extraction and Microbiome (suppl. Methods)

DNA was extracted from stool using the PowerSoil™ DNA HTP extraction kit (Qiagen). Amplicon libraries targeting the V4 region of the 16S rRNA gene were prepared using primers 515F and 806rcbc0 and paired-end sequenced on an Illumina MiSeq. Raw sequences were quality filtered and merged using PEAR (29), then processed with a custom pipeline combining Qiime (30) and Vsearch (31) scripts (Suppl. Methods for more details). Data were rariefied to an equal depth of 9,900 seqs per sample to avoid bias. Metagenomic libraries were prepared from 100-250pg of DNA using the Nextera XT DNA Library Preparation kit (Illumina) and sequenced on a HiSeq 2500 targeting 2.5 Gb of sequence per sample. Demultiplexing and generation of fastq files was performed using the Picard suite (https://broadinstitute.github.io/picard/command-line-overview.html). Quality control and removal of human reads was performed using KneadData (http://huttenhower.sph.harvard.edu/kneaddata). MetaPhlAn2 v2.6.0 (32) was used for taxonomic profiling and HUMAnN2 v 10.0.0 (33) in Uniref90 mode was used for functional analysis. Antibiotic resistance factors were profiled using ShortBRED (34) with markers produced from the Comprehensive Antibiotic Resistance Database. All Picard DNA workflows were performed at the Broad Institute Genomics Platform (details in Supplemental Methods).

Sequence data are available from the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under BioProject PRJNA532645.

Sample size

Based on a previous study we anticipated a mean difference in PUCAI of 20 points between the treatment groups at day 5 with a SD of 13-20 points (35). We thus needed to study 13 children and each group to reject the null hypothesis that the population means are equal with probability (power) 80% and $P<0.05$, and with two dropouts, we aimed at 28 children.

Statistical analyses

Student's t-test or Wilcoxon rank sum test as appropriate were used to compare continuous variables. Categorical data (e.g. remission rates) was compared using Chi square or Fisher's exact, as appropriate. There were 4 children (14%) who were discharged prior to day 5 and two that were withdrawn from the trial due to deterioration. Their day 5 parameters were imputed using the last observation carried forward (LOCF) principle.

Shannon index for microbial diversity and Jaccard/Bray-Curtis distances were calculated using 'vegan' R package. Spearman correlation was used to associate microbial indexes, relative abundances or pairwise distances with PUCAI scores; FDR-correction for multiple hypothesis testing was applied as needed. Kruskal-Wallis or paired Wilcoxon were used to compare microbial indexes between the two treatment groups. ANOSIM (Analysis of Similarities) was used to test for similarities in microbiome composition between the two treatment groups. All microbiome analyses were conducted in R and other analysis in SPSS. Significance level was set at $p<0.05$.

RESULTS

A total of 31 patients were randomized, of whom three were excluded per eligibility criteria due to positive infectious screening within 72 hours (one each: Salmonella, Rota virus and cytomegalovirus (CMV)), leaving 28 children (mean age 13.9±4.1 years and 23 (82%) extensive colitis) in the ITT analysis (Figure 1, Table 1). Eight (29%) were recruited at time of first presentation with colitis, the remainder having an acute severe exacerbation of known chronic UC. There were no differences in any of the baseline clinical characteristics between the 16 children of the AB+IVCS arm and the 12 of the IVCS arm (Table 1). Of the 16 children in the AB+IVCS arm, 12 (75%) received the standard cocktail of vancomycin/amoxicillin/metronidazole/doxycycline, 2 (12.5%) received ciprofloxacin instead of doxycycline because of age (<7 years) and 2 (12.5%) received gentamicin instead of doxycycline because of allergy and discretion of the treating physician due to the age of 5 years.

The mean day-5 PUCAI was lower in the AB+IVCS arm (25±17) than the IVCS arm (40±20; p=0.037), meeting the primary endpoint (Figure 2). There were 3 (19%) children in remission by day 5 in the AB+IVCS arm compared with 1 (8%) in the IVCS arm (p=0.61). Calprotectin was numerally lower in the AB+IVCS vs. the IVCS arm but without statistical significance given the large variability of the calprotectin values and the small sample size (Figure 2). The need for second line medical therapy at discharge and colectomy rate at 1-year were both low and did not differ between the groups (Figure 3), bearing in mind that the sample size had a very low power for this outcome. The time from admission to second line medical therapy in the five children who required it ranged from 6 to 14 days.

PUCAI scores predicted the need for second line medical therapy in those who received infliximab or cyclosporine by discharge both at day 3 (median PUCAI 70 (IQR 60-75) points

vs 35 (20-40), respectively; p=0.016) and day 5 (55 (40-60) vs. 30 (10-37.5), p=0.039). Area under the ROC curve for the PUCAI to predict need for second line therapy was 0.84 (95%CI 0.61-1.0) at day 3 and 0.80 (0.56-1.0) at day 5.

Safety

Eighteen adverse events were recorded in 10 children (63%) from the IVCS+AB group (n=18 events) and 16 were recorded in 7 children of the IVCS group (58%; p=0.9) within 1 month of admission (Suppl. Table 1). Two events in the IVCS+AB group were judged to be possibly related to the intervention: dizziness and allergic rash. Both were non-serious but resulted in withdrawal of the antibiotics and the children considered as failures in the ITT analysis. Besides the one child with mild allergic rash, no other intolerance or allergic reactions to the antibiotics were recorded. There were C. *difficile* infections in both groups throughout the 1-year follow-up of all patients.

Microbiome

Stool for microbiome was collected from 25 children pre-treatment. Based on 16S rRNA gene sequencing, the microbiome of most patients at admission was highly dysbiotic. These pre-treatment samples were characterized by low diversity (median Shannon at admission:2.1 , range 0.3 to 3.2; as compared to samples taken during remission: 2.7, range 1.3 to 3.5; p=0.002) and high relative abundance of Gammaproteobacteria (median relative abundance: 20%, range 0.04% to 97%; compared to remission samples: median 0.02%, range 0.02% to 57%; p=0.0003; see also Figure 4 for family-level and Supplemental Figure 1 for genus-level). In the IVCS arm, microbial diversity at admission strongly correlated with day 5 PUCAI (r=0.72, p=0.01; Figure 5). In contrast, in the IVCS+AB arm, no such association was noted given the antimicrobial effect (Figure 5).

Highly dominant genera, comprising over 33% of the microbiome, were observed in 17 (68%) of the patients before treatment (Table 2). *Escherichia, Haemophilus* and *Enterobacter,* all of which belong to the class Gammaproteobacteria, which rarely dominates the healthy gut microbiome, were abundant in 9 patients (36%), and comprised 40-90% of their microbiome. To determine which species were contributing to this Proteobacteria signal, we generated metagenomic data which allows species-level resolution for taxonomic profiling. Prior to treatment at "day 0", we observed high relative abundance of *Escherichia coli, Klebsiella pneumoniae,* and *Haemophilus parainfluenzae* (Figure 6A).

To identify microbial differences associated with remission, we next compared the microbial profiles of the initial samples to the samples from a patient's first remission time point with a particular focus on Proteobacteria. At first remission, none of the IVCS patients showed Proteobacteria blooms, whereas several patients who received IVCS+AB continued to display Proteobacteria dominance which were also more common in this group at baseline (Figure 6B).

Antibiotic treatment, as expected, exerted a large effect on the microbial population. The microbiome of samples taken during treatment clustered according to treatment type (ANOSIM p=0.001, r=0.67, Suppl. Figure 2A) and displayed an expansion of taxa belonging to the Gammaproteobacteria (mainly *Escherichia* and *Haemophilus*) coupled to a reduction of multiple taxa belonging to the *Clostridiales* and *Bacterodiales* orders (to which most known butyrate producers belong). Accordingly, microbial diversity was strongly reduced during antibiotic treatment. Nevertheless, rapid recovery of the microbiome was observed as early as 2 months after cessation of antibiotic treatment (Suppl. Figure 3). While some patients from

IVCS+AB group tended to higher *Escherichia* levels following treatment, overall post-treatment remission samples did not segregate according to type of treatment received (Suppl. Figure 2B, ANOSIM p=0.2, r=0.03).

A potential concern in the context of antibiotic treatment is the development of antibiotic resistance. We therefore quantified within-patient shifts in the abundance of antimicrobial resistance factors over time (Suppl. Figure 4). Overall, increased resistance against ciprofloxacin, doxycycline and tetracycline was observed by day 30 in patients receiving antibiotics but not in those who received steroids-only. We did not witness a significant increase for amoxicillin or metronidazole. These resistance levels returned to baseline levels after 90 days, with the exception of doxycycline resistance, which remained elevated for up to a year post-treatment.

DISCUSSION

In this first RCT ever conducted in pediatric ASC, we found that an antibiotic cocktail of four oral antibiotics in addition to standard IVCS, was effective in reducing the PUCAI score after 5 treatment days as compared with IVCS alone. Secondary endpoints of remission rate and calprotectin value were numerically better in the intervention arm but did not reach statistical significance in this small study. The need for second-line medical therapy or colectomy was similar between the treatment arms. This pilot study was not powered and did not aim to show a difference in these endpoints. The number of children requiring second line therapy was lower than previously reported in both groups (18% compared with previous reports of 30-35% (36)). Considering the observed ~20% steroid-failure rate and even if the treatment benefit of antibiotics is as high as 30% in avoiding second line treatment (i.e. 14% in the

intervention arm), randomization of 1228 children would be required to show such a difference with a power of 80%. Nonetheless, the effect of antibiotics has previously been inversely correlated with disease activity in pediatric UC (20) and thus the observed added benefit of the cocktail in our trial of severe colitis, may be encouraging.

Using both 16S rRNA gene sequencing data (which allows detection of rare taxa) and metagenomic data (which allows species-level classification), we have examined for the first time pre-treatment microbiome of patients admitted for ASC. Interestingly, two-thirds of children had a dominant bacterial genus at admission, possibly reflecting the reduction in microbial diversity associated with active inflammation. Diarrhea and oxidative stress favor taxa which are fast growing and facultatively aerobic, as are most gut proteobacterial species*;* adhesion and penetration of the gut epithelial barrier have been documented for some strains of the genus *Escherichia,* which was especially dominant at admission (37).

It is tempting to assume that some of the severe steroid-resistant ASC attacks may be secondary to an unknown uncultured infection underlying the severe mucosal inflammation. Indeed, this has been described with CMV (38), cryptosporidium (39) and C. *difficile* (40), that are more commonly seen in patients with acute UC than in the general population. However, in the IVCS+AB group we found no correlation between microbial features at admission and clinical response five days later. An alternative hypothesis is that the broad-spectrum coverage of the Jerusalem cocktail led to an overall decrease in bacterial abundance, rather than targeting particular species.

In contrast to what we observed with the antibiotics-treated group, microbial diversity at admission was negatively correlated with response in the steroid-only treated group. Since a diverse gut microbiome is considered beneficial, this finding may appear paradoxical. However, microbial diversity has been shown to correlate with bacterial loads (41, 42). Thus, patients with high microbial diversity at admission may have larger antigenic exposure and consequently a lower response. This explanation is consistent with the lack of correlation between diversity and response observed in the IVCS+AB treated group, as antibiotics reduce the bacterial load across all the patients.

PUCAI scores predicted the need for second line medical therapy at days 3 and 5 with high accuracy. This reinforces previous findings (35) and ECCO-ESPGHAN guidelines (23) in which second line medical therapy is based on the PUCAI score at these time-points.

There are now ample of data to implicate the microbiome as a main factor in the occurrence of IBD (43). Data to support the role of antibiotics in treating a subset of resistant UC are steadily accumulating, both from RCT's in adults (6, 7) (8, 11) (12, 13) (14) (15) and case series in children (18-20). The diversity of studied regimens and treatment protocols, however, hampers our ability to draw conclusions from the research arena to clinical practice. We thus based our cocktail on previously reported successful experience with the Japanese cocktail and vancomycin, while allowing other antibiotics for allergic and younger children in whom doxycycline is contraindicated. Concerns regarding adverse events reduce the feasibility of using antibiotics for long periods and we show that the resistance was transient for all agents except doxycycline. Moreover, ASC is an acute, often dramatic, scenario with significant morbidity, and a potential benefit might outweigh the risk of drug resistance. Nonetheless, this was a pilot RCT and antibiotics cannot be routinely recommended until

larger studies demonstrate a reduced need for second line treatment or colectomy. Furthermore, our findings on the microbiome during episodes of ASC may facilitate future improvement in the antibiotic cocktail, perhaps towards a more personalized therapeutic approach.

ACKNOWLEDGEMENTS

We thank Ramnik Xavier, Tiffany Poon, Melanie Schirmer, Zivia Shavit, Gili Focht and Moran Yassour for helpful discussions and study conduct and management. We additionally thank the Broad Institute Microbial 'Omics Core and Genomics Platform for generation of sequencing data.

This work was funded by grants from the Helmsley Charitable Trust and the International Organization of IBD (IOIBD) (both to DT), as well as by the Center for Microbiome Informatics and Therapeutics Center for Microbiome Informatics and Therapeutics (Award 6932308 PO# 5710003938) to HV.

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LEGENDS to FIGURES

Figure 1: CONSORT study flowchart

Footnote: ITT, intention to treat.

Figure 2: Pediatric UC activity index (PUCAI) and fecal calprotectin values stratified by the treatment groups

Figure 3: Second line medical and surgical management by discharge (DC) and 1 year (yr).

Footnote: IFX, infliximab; CysA, cyclosporine A.

Figure 4: Microbial dysbiosis at family-level on admission from 16S rRNA gene data

Footnote: Each bar represents the pre-treatment microbiome of a single patient rank-ordered of increasing Gammaproteobacteria relative abundance. Rare families (i.e. <3% across all samples) were excluded. The major gut phyla are color coded: shades of brown for Bacteroidetes; purple, green and blue for Lactobacillales, Clostridiales, and Erysipelotrichales orders of the Firmicutes phylum; Proteobacterial families are in shades of red.

Figure 5: Correlation between microbial diversity at admission and PUCAI score on day 5.

Figure 6: Proteobacterial species abundance from metagenomic data per participant prior to treatment (day 0) and on the day of first remission. Proteobacteria at < 5% abundance were categorized as “Other Proteobacteria”.

Suppl. Figure 1: Microbiome at genus-level on admission from 16S rRNA gene data. Each bar represents the pre-treatment microbiome of a single patient rank-ordered of increasing relative abundance of Gammaproteobacteria

Footnote: Rare genrea (i.e. <3% across all samples) were excluded. The major gut phyla are color coded: shades of brown for Bacteroidetes; purple, green and blue for Lactobacillales, Clostridiales, and Erysipelotrichales orders of the Firmicutes phylum; Proteobacterial families are in shades of red.

Suppl. Figure 2: Clustering of samples according to 16S rRNA gene microbial composition during and after treatment (A) samples taken during treatment; (B) samples taken during remission (i.e. PUCAI<10) and at least 2 months after treatment.

Footnote: Principle Coordinate Analysis used for dimension reduction and visualization of Bray-Curtis distance matrix. Similar results obtained if using Jaccard distance matrix (during-treatment ANOSIM p-value=0.001, r=0.44; post-treatment ANOSIM p-value=0.4, r=0).

Suppl. Figure 3: Microbial features across time in both treatment groups based on 16S rRNA gene sequencing data. A: Shannon diversity index; B: *Clostridiales* order (relative abundance); C: Gammaproteobacteria class; D: *Escherichia* (relative abundance).

Footnote: Time-points: T0: admission, T1: during treatment; T2-T4: post-treatment (T2: >21 & <60 days; T3:>60 & <180 days,T4: >180 days & <1 year).

Suppl. Figure 4: Fold change in antimicrobial resistance factors relative to baseline.

Footnote: The sum of resistance factors was calculated for each of the four antibiotics for each sample, respectively. Within each participant, log fold change between day 0 antibiotic resistance and antibiotic resistance in the follow-up sample was then calculated. A fold

change of 0 indicates that resistance to a given antimicrobial did not change within a participant relative to baseline.

TABLES

Table 1: Baseline characteristics of enrolled children at admission. Counts (%), medians (interquartile range) or means ± SD are presented as appropriate for the data distribution. **None of the variables were significantly different between the groups.**

	Entire cohort (n=28)	***AB+IVCS (n=16)***	***IVCS (n=12)***
Males	15 (47%)	10 (63%)	5 (42%)
Age (years)	13.9±4.1	13.5 ± 4.7	14.6 ± 3.3
Range (years)	4.4-18	4.4-18	8.6-18
Disease duration (months)	7 (0-20)	11 (0.5-20)	.25 (0-23)
First attack	8 (29%)	4 (25%)	4 (33%)
Exacerbation	20 (71%)	12 (75%)	8 (67%)
Disease extent			
Left sided (E2)	5 (18%)	4 (25%)	1 (8%)
Extensive/pancolitis (E3+E4)	23 (82%)	12 (75%)	11 (92%)
PGA of disease activity (mm)	75 ± 9	77 ± 10	73 ± 6
PUCAI at admission	74 ± 7	71 ± 12	75 ± 7
# severe (i.e. ≥65points)	28 (100%)	16 (100%)	12 (100%)
Fecal calprotectin	1780 (1188-2890)	1733 (1125-2890)	1840 (1433-2825)
C-reactive protein (mg/dL)	1.0 (0.6-2.8)	0.73 (0.4-2.0)	1.2 (0.7-3.2)
ESR (mm)	27 (17-46)	33 (16-50)	22 (18-33)
Albumin (mg/L)	3.8 (3.4-4.1)	3.7 (3.3-4.1)	4 (3.7-4.1)
Hemoglobin (mg/L)	11.9 (10.3-12.8)	11.3 (9-11.8)	12.3 (11.2-12.8)
Treatment at admission			
Oral prednisone	7 (25%)	3 (19%)	4 (33%)
Thiopurine	6 (21%)	3 (19%)	3 (25%)
Anti-TNF	4 (14%)	3 (19%)	1 (8%)

PUCAI, Pediatric Ulcerative Colitis Activity Index; PGA, physicians' global assessment;

ESR, erythrocyte sedimentation rate

Table 2: Genera which compromised over 33% of the microbiome at admission

Dominating genera	***Number of patients***	***Relative abundance (Range)***	***Phylum***
Bacteroides	6	0.34-0.61	Bacteroidetes
Parabacteroides	1	0.43	Bacteroidetes
Faecalibacterium	1	0.57	Firmicutes
Enterobacter	1	0.63	Proteobacteria *(class: Gammaproteobacteria)*
Escherichia-Shigella	6	0.4-0.97	Proteobacteria (*class: Gammaproteobacteria)*
Haemophilus	2	0.57-0.78	Proteobacteria *(class: Gammaproteobacteria*)

Suppl. Table 1: Safety - Adverse Events

	AB+IVCS (n=5 AE in 4 children)	***IVCS (n=6 AE in 5 children)***
Non-serious adverse events (AE)[a]	4 (25%)	2 (16.7%)
Nausea	1 (6.3%)	0 (0%)
Dizziness/headache	1 (6.3%)	1 (8.3%)
Arthralgia	0 (0%)	1 (8.3%)
Treatment reactions	2 (12.5%)[b]	0
Serious adverse events (SAE)[a]	1 (6.3%)	4 (33.3%)
Disease exacerbation	1 (6.3%)	2 (16.7%)
Vomiting	0 (0%)	1 (8.3%)
Infections	0 (0	1 (8.3%)

[a] None of the SAE were judged as drug-related; 3 AEs were likely/possibly related to the treatment

[b] One was a treatment reaction to infliximab, the other one to the antibiotics

SUPPLEMENTARY DATA

Microbiome methods

DNA extraction, 16S rRNA amplicon and metagenomic data generation

DNA was extracted from thawed stool samples using the DNeasy PowerSoil HTP 96 kit (Qiagen product number 12955-4). 16S rRNA gene libraries targeting the V4 region of the 16S rRNA gene were prepared by first normalizing template concentrations and determining optimal cycle number by way of qPCR. Using primers 515F and 806rcbc0. To ensure minimal over-amplification, each sample was diluted to the lowest concentration sample, amplifying with this sample optimal cycle number for the library construction PCR using the Golay primer set (Caporaso, 2011 and 2012). Four replicates were pooled, cleaned and normalized prior to sequencing on an Illumina MiSeq in paired-end mode (2x175bp) using custom index 5'-ATTAGAWACCCBDGTAGTCC GG CTGACTGACT-3' and custom Read 1 (5'-TATGGTAATT GT GTGYCAGCMGCCGCGGTAA-3') and Read 2 (5'-AGTCAGTCAG CC GGACTACNVGGGTWTCTAAT-3') primers.

515F: 5'-
AATGATACGGCGACCACCGAGATCTACACTATGGTAATTGTGTGCCAGCMGCCGCGGTAA-3'

806rcbc0:

5'-
CAAGCAGAAGACGGCATACGAGATTCCCTTGTCTCCAGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3'

For metagenomic library construction, DNA samples were quantified by Quant-iT PicoGreen dsDNA Assay (Life Technologies) and normalized to a concentration of 50pg/uL. Libraries were prepared from 100-250pg of DNA using the Nextera XT DNA Library Preparation kit (Illumina) according to the manufacturer's protocol, with reaction volumes scaled accordingly. Insert sizes and concentrations for each library pool of 96 samples were determined using an Agilent Bioanalyzer DNA 1000 kit (Agilent Technologies). Libraries were sequenced at the Broad Institute Genomics Platform on a HiSeq 2500 targeting 2.5 Gb of sequence per sample with 101 bp paired-end reads. Post-sequencing de-multiplexing and generation of BAM and Fastq files are generated using the Picard suite

(https://broadinstitute.github.io/picard/command-line-overview.html).

Taxonomic and functional profiling of metagenomic data

The KneadData pipeline was used to quality control raw sequencing reads. Briefly, the pipeline first quality trims the 3' ends of reads using Trimmomatic and then filters out all reads <60 nt in length. Reads mapping to human reference genome hg19 using bowtie2 are then removed. Taxonomic profiling of collected stool samples was performed using MetaPhlAn2 v2.6.0. MetaPhlAn2 maps reads to marker genes unique to a given microbial clade in order to estimate the abundance of that clade. In this analysis, we considered the resulting abundance profiles at species level.

Functional profiling for metagenomic features was performed using HUMAnN2 v 10.0.0 in Uniref90 mode. HUMAnN2 first maps metagenomic reads to the pangenomes of species identified by MetaPhlAn2. Coding sequences in pangenomes are assigned to UniRef90 families. Reads failing to map to a pangenome are then mapped to UniRef90 via translated search using DIAMOND. Hits are

scored by alignment quality, sequence length, and sequence coverage. Gene-level outputs are given in reads per kilobase units and stratified by species contribution. Gene abundances can be mapped to other functional annotation systems based on UniProt annotations. In this study, genes were remapped to their corresponding Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthologies. KEGG Orthologs were then grouped into their corresponding modules by total sums.

Samples were excluded from analysis if fewer than 5,000,000 reads were present in the sample after QC filtering and removal of human reads. Metagenomically determined species were considered absent from a sample if their abundance was less than 0.25% of the sample or if the species was present in fewer than two samples across the cohort.

Antibiotic resistance factor profiling

Antibiotic resistance factors were profiled using ShortBRED with markers produced from the Comprehensive Antibiotic Resistance Database. Briefly ShortBRED produces family-level markers by finding highly conserved sequence regions within orthologous gene families and then quantifies reads aligning to these makers, normalizing by the length of the marker.

Pseudocounts of one half of the minimum non-zero observed value of a feature were added to each observation of zero feature abundance per antimicrobial resistance feature as quantified by shortBRED.

shortBRED marker family determination: resistance factors for 6 antibiotics were determined by parsing through all CARD ontologies with a particular focus on the relational keywords “confers_resistance_to:” and references to any of these antibiotics.

Doxycycline

'ARO:3000186', 'ARO:3000190', 'ARO:3000191', 'ARO:3000192', 'ARO:3000193', 'ARO:3000194', 'ARO:3000195', 'ARO:3000196', 'ARO:3000197', 'ARO:3000205', 'ARO:3000556', 'ARO:3004470'

Vancomycin

'ARO:3000236', 'ARO:3002905'

Ciprofloxacin

'ARO:3000386', 'ARO:3000391', 'ARO:3000419', 'ARO:3000421', 'ARO:3000448', 'ARO:3000753', 'ARO:3000769', 'ARO:3000822', 'ARO:3001327', 'ARO:3002547', 'ARO:3002790', 'ARO:3003032', 'ARO:3003038', 'ARO:3003294', 'ARO:3003295', 'ARO:3003296', 'ARO:3003297', 'ARO:3003298', 'ARO:3003304', 'ARO:3003305', 'ARO:3003306', 'ARO:3003307', 'ARO:3003308', 'ARO:3003309', 'ARO:3003310', 'ARO:3003311', 'ARO:3003312', 'ARO:3003315', 'ARO:3003316', 'ARO:3003317', 'ARO:3003684', 'ARO:3003789', 'ARO:3003817', 'ARO:3003818', 'ARO:3003844', 'ARO:3003921', 'ARO:3003926', 'ARO:3003939', 'ARO:3003947', 'ARO:3003964', 'ARO:3004048', 'ARO:3004051', 'ARO:3004052', 'ARO:3004053', 'ARO:3004066', 'ARO:3004068', 'ARO:3004078', 'ARO:3004079', 'ARO:3004080', 'ARO:3004083', 'ARO:3004103', 'ARO:3004126', 'ARO:3004128', 'ARO:3004468'

Amoxicillin

'ARO:3000873', 'ARO:3000900', 'ARO:3001396', 'ARO:3001397', 'ARO:3003041', 'ARO:3003042', 'ARO:3003043', 'ARO:3003562', 'ARO:3004124', 'ARO:3004359'

Metronidazole

'ARO:3003950'

Tetracycline

'ARO:3000026', 'ARO:3000165', 'ARO:3000166', 'ARO:3000167', 'ARO:3000168', 'ARO:3000173', 'ARO:3000174', 'ARO:3000175', 'ARO:3000177', 'ARO:3000178', 'ARO:3000179', 'ARO:3000180', 'ARO:3000181', 'ARO:3000182', 'ARO:3000183', 'ARO:3000186', 'ARO:3000190', 'ARO:3000191', 'ARO:3000192', 'ARO:3000193', 'ARO:3000194', 'ARO:3000195', 'ARO:3000196', 'ARO:3000197', 'ARO:3000205', 'ARO:3000343', 'ARO:3000373', 'ARO:3000384', 'ARO:3000386', 'ARO:3000464', 'ARO:3000476', 'ARO:3000478', 'ARO:3000481', 'ARO:3000556', 'ARO:3000561', 'ARO:3000565', 'ARO:3000566', 'ARO:3000567', 'ARO:3000569', 'ARO:3000572', 'ARO:3000573', 'ARO:3000770', 'ARO:3000771', 'ARO:3000772', 'ARO:3000797', 'ARO:3000799', 'ARO:3001328', 'ARO:3002870', 'ARO:3002871', 'ARO:3002892', 'ARO:3002893', 'ARO:3002894', 'ARO:3003029', 'ARO:3003032', 'ARO:3003038', 'ARO:3003054', 'ARO:3003062', 'ARO:3003196', 'ARO:3003410', 'ARO:3003411', 'ARO:3003499', 'ARO:3003510', 'ARO:3003694', 'ARO:3003697', 'ARO:3003964', 'ARO:3003980', 'ARO:3003981', 'ARO:3004031', 'ARO:3004034', 'ARO:3004051', 'ARO:3004052', 'ARO:3004053', 'ARO:3004062', 'ARO:3004076', 'ARO:3004078', 'ARO:3004079', 'ARO:3004080', 'ARO:3004128', 'ARO:3004441', 'ARO:3004442', 'ARO:3004470'