Volume 10:1-11

UJEMI+

The Undergraduate Journal of Experimental Microbiology & Immunology (+Peer Reviewed)

Surgical Intervention Correlates with Reduced Bacterial Diversity in the Gut Microbiome of Crohn's Disease Patients Exhibiting Low Levels of Inflammation

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SUMMARY Crohn's Disease is a type of inflammatory bowel disease characterized by dysbiosis and chronic inflammation of the gastrointestinal tract. First line therapies utilize anti-inflammatory treatments alongside endoscopies to manage and monitor disease course. Biomarkers such as fecal calprotectin are being investigated to measure inflammation and predict disease recurrence, and offer a less invasive method to track disease progression. Surgical resection serves as a last resort treatment to medical therapies, but this alternative has been shown to alter microbial diversity post-operatively. This study aims to identify the link between inflammation and microbial composition in Crohn's Disease patients with or without surgical resection. Diversity analysis revealed reduced alpha diversity in Crohn's Disease patients following surgical intervention. This observation was minimal in patients with pre-existing inflammation compared to those without inflammation. In parallel, we ran the same diversity analysis based on disease severity. We found that inflammation masked the reduction in alpha diversity in patients with more severe disease, consistent with the effects of inflammation on surgical resection. Additionally, indicator species analysis revealed reduced abundance of anti-inflammatory taxa in patients following surgical resection. These findings provide insight into the post-operative intestinal environment, and can help inform post-operative care and limit significant alterations to the gut microbiome in an already dysbiotic environment.

INTRODUCTION

Patients living with Crohn's Disease experience an array of symptoms including chronic abdominal pain, diarrhea, and bowel obstruction that require long-term treatment to prevent flare ups, disease progression, and intestinal complications (1). The first line of treatment utilizes medical therapies such as immunotherapy and small molecules to induce and maintain disease remission, and improve the quality of life in patients (2, 3). However, 3 in 4 Crohn's Disease patients require surgery at some point in their lives, many of those needing it within 20 years of diagnosis (1, 3). Surgical intervention is the last resort treatment typically for patients who are not responsive to medical therapies, but it is not a curative treatment, with disease recurrence occurring in approximately 30% of patients (3, 4).

It has been established that the gut microbiome plays a key role in maintaining host homeostasis including nutrition and immune metabolism, and the synthesis of various metabolites (5). Crohn's Disease, a type of inflammatory bowel disease (IBD), has been shown to alter the intestinal microbial composition in its patients compared to healthy individuals (6). Crohn's Disease has been shown to be associated with reduced abundance of species from the Firmicutes phylum and increased abundance of Proteobacteria (4–7). While surgical intervention as a treatment option can improve the quality of life of patients, surgery

Published Online: September 2024

Citation: Bremner, Feng, Forward, Guan, Zhang. 2024. Surgical intervention correlates with reduced bacterial diversity in the gut microbiome of Crohn's Disease patients exhibiting low levels of inflammation. UJEMI+ 10:1-11

Editor: Shruti Sandilya and Ronja Kothe, University of British Columbia

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causes both physical and physiological changes to the gastrointestinal tract (8). These changes can impact factors such as the gastrointestinal surface area, acidity, and intestinal transit, and have the potential to shift the microbiome composition (8).

Prior studies have shown that bowel resection in Crohn's Disease patients have a large effect on the gut microbiome that varies between cohorts of patients (9). Strömbeck et al. observed distinct microbiome compositions between patients with disease relapse and patients in remission post-operatively (4). Identification of specific taxa associated with postoperative disease course can help physicians non-invasively identify patients at-risk for disease relapse that may require a more intense therapeutic regimen (4). Specifically, *Alistipes*, a genus of bacteria, was found to be higher in abundance in patients in remission compared to patients with disease relapse (4). However, little is understood regarding the differences in microbiome between Crohn's Disease patients with or without surgery.

Additionally, inflammation has been shown to have significant effects on health outcomes. In addition to contributing to dysbiosis, a hallmark of inflammatory bowel disease, inflammation has been shown to be associated with cancer and surgical outcomes (10). One study found that moderate inflammation based on serum C-reactive protein, a biomarker, was a predictor of cancer-related death following surgical intervention (11). Assessing inflammation using biomarkers offer a minimally invasive approach to diagnose and evaluate predict disease activity (12). Calprotectin is an antimicrobial protein secreted by neutrophils used as a marker to assess inflammatory activity that may predict postoperative disease recurrence (12, 13). Calprotectin can be measured from fecal samples and has been shown to be more accurate compared to traditional inflammatory markers like C-reactive protein (12).

Given the significant role of the gut microbiome in the context of Crohn's Disease, particularly post-surgery, changes in microbiome composition are being studied as potential indicators of disease progression. Non-invasive methods such as microbiome composition and fecal calprotectin have been studied individually as potential predictors of disease recurrence post-surgical intervention (12). Microbiome compositions can be measured using alpha diversity metrics, which assess species diversity within a single environment (14). Two key metrics are Chao1, which focuses on species richness and rare species detection, and the Shannon index, which balances both richness and evenness by considering species numbers and their relative abundances (14). Though reduced alpha diversity has been demonstrated in postoperative patients with IBD, the effects of inflammation and surgery on the microbiome have been poorly studied (9). Since surgical intervention can affect the disease course in patients and may play a role in post-operative complications, more research is still needed to understand the relationship between the microbiome and surgical outcomes (15).

In this study, we aim to identify the relationship between inflammation and the microbiome in patients with Crohn's Disease with or without surgical resection using fecal calprotectin as a biomarker for inflammation and amplicon sequencing data collected by Halfvarson et al. (16). As IBD patients in remission have a higher abundance of bacterial species compared to those in active states marked by inflammation, we hypothesize that microbial diversity will be reduced in the gut with inflammation and following surgery. Our findings can help elucidate predictors associated with surgery based on inflammatory status, and may assist physicians in identifying patients at risk of disease recurrence.

METHODS AND MATERIALS

Data availability

All the data and scripts can be found in the GitHub repository via the following link**:** https://github.com/Andyrooooo16/MICB475-Team-13

Dataset overview. The 16S rRNA sequencing data was collected over the course of 2 years from 683 fecal samples belonging to 137 participants in an inflammatory gut microbiome study conducted by Halfvarson et al. (16). For this study, the 137 participants were filtered in R v 4.2.3 to include only those with Crohn's Disease (n=48) and healthy controls (n=9) (16, 17).

Preprocessing and wrangling of the metadata. The raw metadata provided by Halfvarson et al. contained 683 fecal samples from 137 participants. We wrangled in R to only include samples collected at the first timepoint to mitigate the impact of participant dropout from subsequent collection timepoints . In the study by Halfvarson et al., calprotectin levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) and used as biomarkers for inflammation (13). In this study, active inflammation is characterized by calprotectin levels exceeding 150 µg/g (13). Based on this threshold, we classified participants in our dataset into two categories: those with Inflammation ('TRUE') (≥ 150 µg/g) and those without Inflammation ('FALSE') (< 150 µg/g). The dataset from Halfvarson et al. includes a column indicating Crohn's Disease behavior, categorized as penetrating (B3), stricturing (B2), or non-stricturing non-penetrating (B1) based on the Montreal classification system (3, 16, 18). B1 represents the least severe form of the disease, B2 a moderate severity, and B3 the most severe (3, 16, 18). Accordingly, we have reclassified this data into 'Low', 'Medium', and 'High' severity levels, corresponding to B1, B2, and B3, respectively. We then combined inflammation and surgical status to create 5 unique parameters: 'Healthy Control', 'inflammation_with_surgery', 'inflammation_no_surgery', 'no_inflammation_with_surgery', 'no_inflammation_no_surgery'. Workflow of the data wrangling can be seen in Script #1.

QIIME2 processing of the dataset. Sequencing data was demultiplexed and quality filtered in QIIME2, subsequently the DADA2 plugin was used to denoise the data (19, 20). Halfvarson et al. used primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) detailed in the protocol by Caporaso et al. to amplify the V4 region of the 16s rRNA gene and generated Amplicon Sequence Variants (ASVs). (16, 21). Amplified sequences were trimmed at the 99th nucleotide during denoising to maintain consistent quality across the reads and maintain an average phred quality score over 30 (19, 22). Taxonomic information was assigned to the ASVs using the naive Bayes classifier in QIIME2 trained on the SILVA 138-99 database (19, 23). Mitochondrial and chloroplast sequences were filtered out of the data in QIIME2 (19). Using MAFFT and FastTree2, the sequences of the ASVs were aligned to generate a phylogenetic tree on QIIME2 (19, 24, 25). Workflow of the QIIME2 processing can be seen in Script #2.

Alpha diversity analyses. Using the processed Halfvarson et al. dataset, we generated an alpha rarefaction curve to determine an optimal sampling depth of 124392. This sampling depth allowed us to retain 5 or more samples for each category within the 'inflammation_with_surgery' column, while allowing all samples to reach their diversity plateau, where additional sampling no longer increases observed species diversity. Our phyloseq object was generated in R using the 'phyloseq' R package, and was rarefied to our chosen sample depth as seen in Script #3 (17, 26). A new column, 'DiseaseSev_with_inflammation' was added to the phyloseq object prior to diversity analysis. Chao1 and Shannon diversity metrics were used to compare differences in richness and abundance of Crohn's Disease patients across different disease parameters. The three primary parameters investigated were: disease severity, surgical status, and inflammatory status. All diversity analyses were compared to healthy controls.

Core microbiome analysis on surgical and inflammatory experimental groups. The core microbiome analysis was conducted using the 'phyloseq', 'microbiome', and 'ggVennDiagram' packages in R (17, 26–28). This analysis was conducted on non-rarefied data without a healthy control. Our detection parameter was set to 0 and our prevalence parameter to 0.7 to be inclusive of potential rare ASVs unique to certain samples. A four-ellipse Venn diagram was created to represent the shared ASVs between the 'Inflammation with Surgery', 'Inflammation without Surgery', 'No Inflammation with Surgery', and 'No Inflammation without Surgery' patient groups.

Indicator species analysis on surgery and inflammation status. We performed indicator species analysis using the following categories: 'Healthy Control', 'Inflammation with Surgery', 'Inflammation without Surgery', 'No Inflammation with Surgery', and 'No

Inflammation without Surgery'. Using the 'indicspecies' package in R, we calculated the significance of each ASV, filtering out those with P-values greater than 0.05 (29). Further, to refine our dataset, we retained only ASVs demonstrating an indicator value greater than 0.87. The resulting significant ASVs were then merged with their taxonomic information. Mean abundances of these ASVs were computed and visualized on a bubble plot using 'ggplot2' (30).

Statistical analysis. For alpha diversity, because the data is not normal, pairwise comparisons were performed using the Wilcoxon rank sum test in R (17). Indicator species analysis was performed with R package 'indicspecies' involving the calculation of the Indicator Value and permutation testing to generate P-values (28). For all statistical tests performed, a p-value of less than 0.05 was considered statistically significant. Significant results were represented on graphs using R package 'ggPubr' (31).

RESULTS

Surgery reduced microbial alpha diversity in Crohn's Disease patients exhibiting low levels of calprotectin. Since inflammatory bowel disease is associated with gut dysbiosis and surgical resection has been associated with reduced microbial diversity, we wanted to analyze the relationship between microbial diversity, inflammation, and surgical status in Crohn's Disease patients (9). Crohn's Disease patients were assigned an inflammatory status if their fecal calprotectin levels exceeded 150 µg/g (n = 23), and were otherwise assigned to a non-inflamed group (n = 25). This classification is not absolute and it should be noted that patients classified into the non-inflamed group have minimal to low levels of inflammation rather than an absolute absence. Shannon and Chao1 diversity metrics were calculated for both patients and healthy controls (n = 9) and similar trends were observed across both metrics (Fig. 1A and B). As healthy controls and non-inflamed Crohn's Disease patients without surgery shared similar levels of diversity, all further analyses will be measured against these

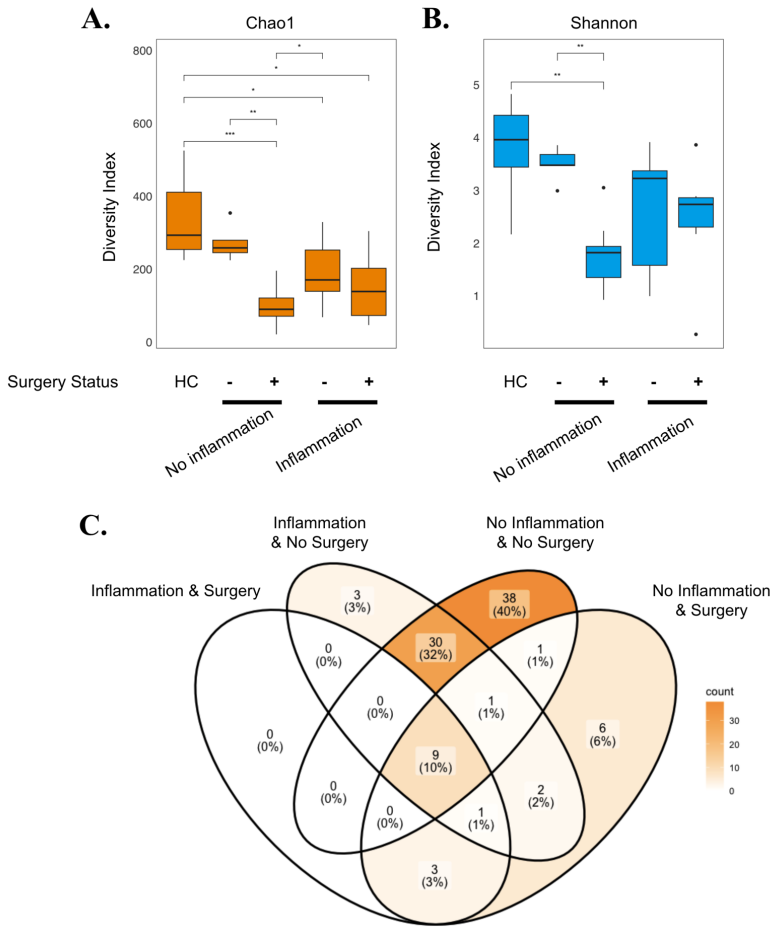

FIG. 1 Surgery reduces microbial diversity in Crohn's Disease patients with no inflammation. (A, B) Chao1 and Shannon diversity metrics were used to measure the impact of surgery on diversity of Crohn's Disease patients with or without surgery compared to healthy controls (HC). Statistical analysis was performed using pairwise Wilcoxon test between all groups, and significant results are indicated by an asterisk (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). **(C)** Core microbiome analysis to determine shared and unique ASVs between Crohn's Disease patients with different inflammatory and surgery statuses. Prevalence and detection cutoffs were 0.7 and 0 respectively.

groups. We observed a significant reduction in alpha diversity in non-inflamed patients following surgical resection relative to the healthy cohort and non-inflamed patients without surgical resection, suggesting that surgical resection may be associated with reduced alpha diversity. Relative to healthy controls, patients with inflammation had reduced alpha diversity regardless of surgical status. Notably, the Chao1 metric revealed a significant reduction in alpha diversity when comparing inflamed patients with surgical resection to healthy controls. No significant reduction in alpha diversity was observed when comparing inflamed patients with or without surgical resection. Overall, this suggests that richness and abundance are reduced following surgical resection but may not be as easily perturbed in inflamed individuals.

Non-inflamed Crohn's Disease patients without surgery showed a more unique microbiome. We performed a core microbiome analysis to further assess the impact of either surgery or inflammation on reduced microbial diversity in patients (Fig. 1C). This analysis revealed the greatest species richness in non-inflamed patients without surgery. However, in the presence of inflammation or following surgical intervention, we see a reduction in the number of unique species. This seems to suggest that surgery may have the strongest association with reduced microbial diversity.

Inflammation confounded reduction of alpha diversity based on disease severity. Chao1 and Shannon diversity metrics were run on Crohn's Disease patients, categorized by disease severity using the Montreal classification (4, 6). Patients were grouped into 'High' (n = 7), 'Medium' (n = 19) or 'Low' (n = 22) levels of disease severity and compared against healthy controls (n = 9) (Fig. 2A and B). Both metrics revealed a consistent trend: "as disease

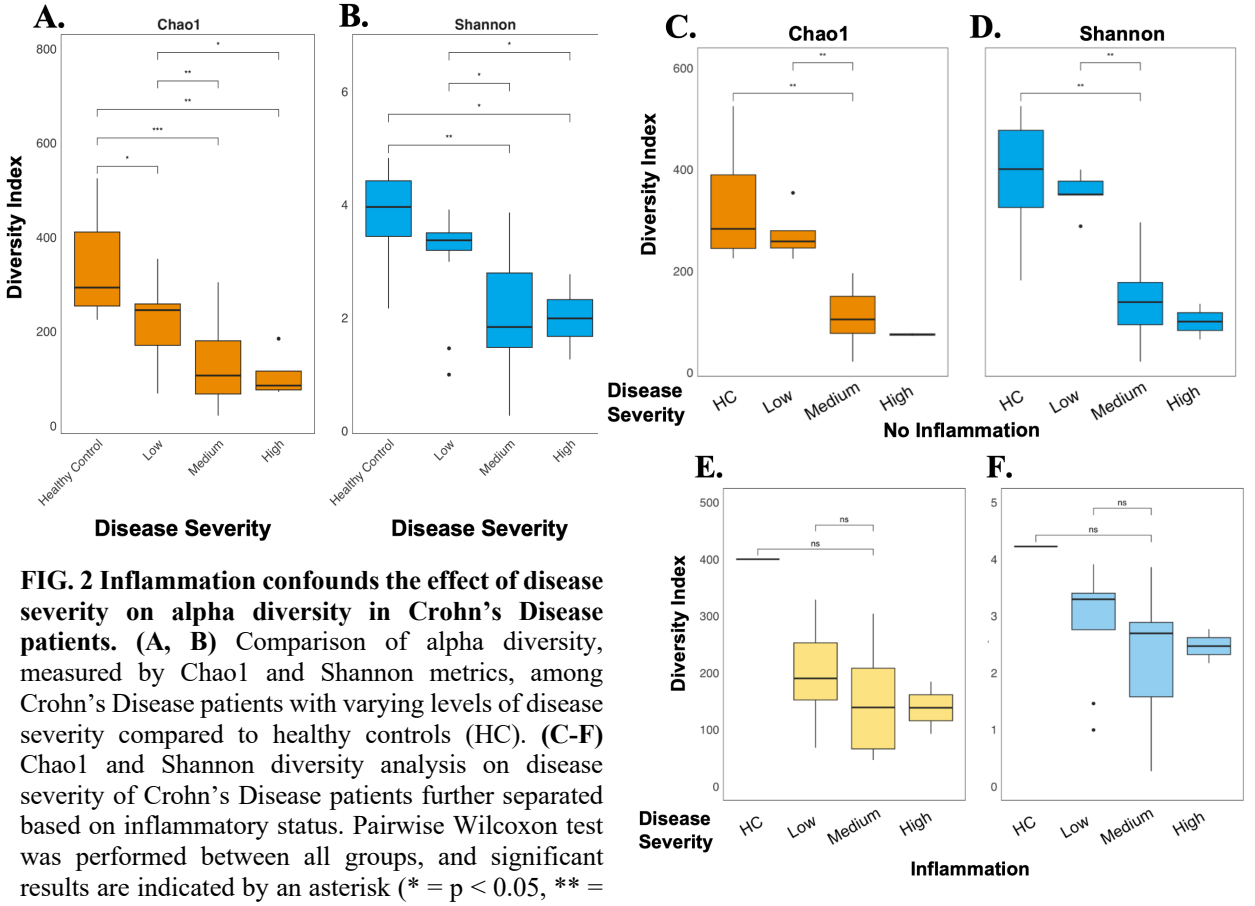

FIG. 2 Inflammation confounds the effect of disease severity on alpha diversity in Crohn's Disease patients. (A, B) Comparison of alpha diversity, measured by Chao1 and Shannon metrics, among Crohn's Disease patients with varying levels of disease severity compared to healthy controls (HC). **(C-F)** Chao1 and Shannon diversity analysis on disease severity of Crohn's Disease patients further separated based on inflammatory status. Pairwise Wilcoxon test was performed between all groups, and significant results are indicated by an asterisk (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

severity increases, alpha diversity reduces. This aligns with previous findings indicating a negative correlation between microbial diversity and disease severity (21). We then investigated whether inflammation had an impact on this trend, repeating the same analyses, grouping patients by inflammation status and disease severity (Fig. 2C-F). Similar to Fig. 2A

and B, an observed reduction of diversity is seen in non-inflamed patients as disease severity worsens, with the greatest loss of diversity occurring in 'Medium' and 'High' disease severity patients relative to the healthy control. Intriguingly, this observation was absent in patients with inflammation, suggesting that inflammation may confound the effect of disease severity on reducing alpha diversity of the gut microbiome.

Taxa associated with short chain fatty acid production were reduced in Crohn's Disease patients following surgical intervention. Indicator species analysis was performed on Crohn's Disease patients to identify taxa unique to various groupings of inflammatory and surgical statuses (Fig. 3). While no specific taxa were exclusive to any single group, all taxa were indicated in some combination of various inflammatory and surgical statuses. Interestingly, a pattern was observed where many of these taxa were absent in either of the groups receiving surgical intervention, including *Faecalibacterium,* and *Oscillospiraceae*. Some taxa were only absent in inflamed patients with surgery such as *Roseburia*, whereas *Prevotella* was only absent in non-inflamed patients with surgery. These highlighted taxa are associated with short chain fatty acid production (32–34).

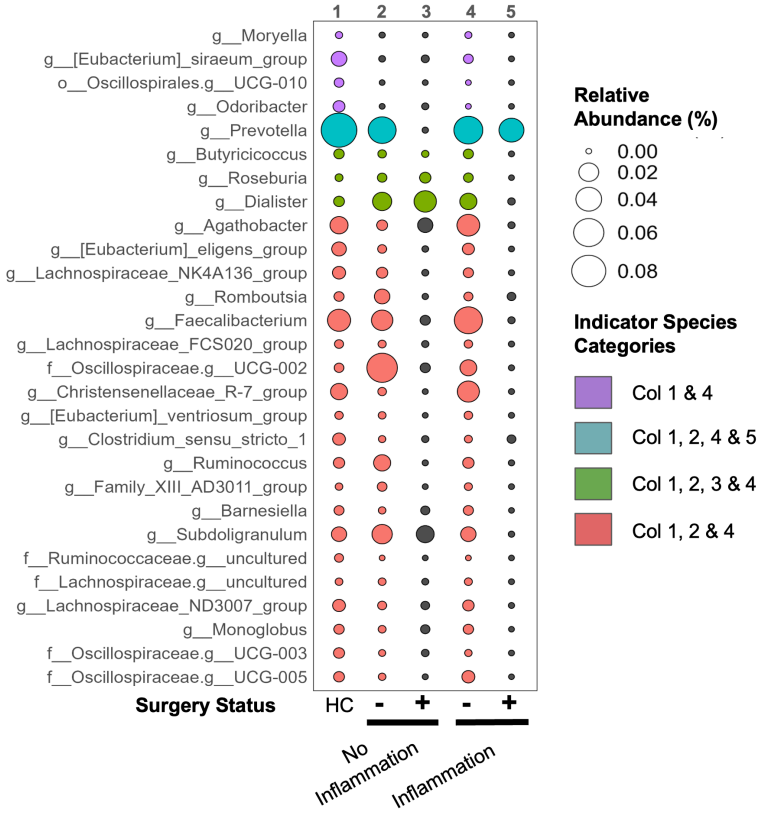

FIG. 3 Key anti-inflammatory microbial species are reduced in Crohn's Disease patients following surgery. Indicator species analysis was performed on Crohn's Disease patients grouped by inflammatory and surgical status. Bubble size represents the relative abundance of a specific bacterial species. While, color indicates species that are statistically significant ($p < 0.05$) for a particular health status. Columns are numbered (top) by inflammatory and surgical status (bottom). Colors indicate taxon groupings in column arrangements.

DISCUSSION

This study explored the link between gut microbiome composition, inflammatory state, and disease severity in Crohn's Disease patients. We leveraged previously published data by Halfvarson et al. considering both surgical history and the presence or absence of inflammation (5).

Alpha diversity is often used to study gut microbiota by measuring the richness and evenness of species within a single sample (29). The results of both our Chao1 and Shannon diversity indices show a significant reduction of microbial diversity in non-inflamed Crohn's Disease patients with surgery compared to non-inflamed patients without surgery (Fig.1). This is consistent with existing literature which have demonstrated that intestinal surgeries decrease the diversity of both gut microbiome and metabolome in patients with existing IBD (9). However, we did not observe a significant reduction in alpha diversity in patients with inflammation that had undergone surgery. This observation suggests that taxa associated with inflammation may be more resilient and not as easily perturbed by changes to the gut

environment such as surgery. It has been shown that the dysbiosis in IBD is also driven by an increase in abundance of pro-inflammatory taxa, particularly species from the Proteobacteria phylum (35). Such species include *Campylobacter concisus* and adherent-invasive *Escherichia coli* (AIEC) (36). In particular, the adhesion abilities of AIEC enables this species to colonize the mucosal lining in the gut, enabling resistance to changes of the intestinal environment (37).

Our data revealed that microbial alpha diversity decreases as disease severity worsens, consistent with literature findings that microbial diversity negatively correlates with disease severity (Fig. 2A, B) (38). Interestingly, patients with inflammation did not have a significant reduction in species richness and abundance as disease severity worsened, which could suggest that inflammation masks the effect of disease severity on microbial diversity (Fig. 2C-F). This aligns with our findings in patients with inflammation that underwent surgery. It is possible that inflammation might alter the gut environment in a way that favors bacterial communities which are less diverse yet more resilient to extensive changes of the intestinal environment in Crohn's Disease patients (39). There are several possible explanations for this phenomenon. Firstly, inflammation reduces the production of mucous which may create a harsher environment that enables more resilient strains of bacterial communities to thrive despite worsening disease severity (40). Secondly, inflammation can activate the immune system which could lead to existing beneficial bacteria in the gut microbiota being disturbed. This reduction could allow harmful bacteria to then colonize the environment (41). By investigating the specific mechanisms by which inflammation modulates the gut microbiome and how these changes might influence disease progression, we may be able to achieve more sustainable remission and improved patient outcomes.

Our indicator species analysis revealed a significant reduction in indicator species following surgical resection, regardless of inflammation status (Fig. 3). Notably, there was an absence of crucial bacterial genera including *Prevotella, Roseburia,* and *Faecalibacterium*, known for their anti-inflammatory properties (36, 37, 42). These genera, especially *Roseburia* and *Faecalibacterium*, play a role in producing butyrate, a vital short-chain fatty acid (SCFA) implicated in Crohn's Disease pathology (36–38, 42). Butyrate and other SCFAs inhibit inflammatory responses by suppressing adhesion molecule and chemokine production, thereby reducing macrophage and neutrophil infiltration (38). Since over 90% of Crohn's Disease patients experience endoscopic signs of recurrence post-surgery, and almost half develop symptoms within three years, the absence of these key genera post-surgery might explain postoperative recurrence in Crohn's Disease patients (39, 40). Furthermore, following surgical intervention, diversity reduction was less significant in patients with pre-existing inflammation compared to those without inflammation (Fig. 1A and B). This aligns with previous research showing a significant reduction in gut microbial diversity post-surgery in Crohn's Disease patients (43). These findings suggest that surgical intervention in Crohn's Disease patients could inadvertently alter the composition of the gut microbiome, potentially impacting the production of short-chain fatty acids and exacerbating the underlying inflammatory condition.

Limitations A limitation of this study was the potential impact of the time lapse between surgical resection and sample collection. As we lack clear insight into this timeframe, it is plausible that the duration could introduce confounding variables, possibly affecting the observations we made in the study. Considering the dynamics of the microbiome over time and throughout the course of the disease, it is plausible that some of our findings may be influenced. For instance, the timing between surgical resection and sample collection is crucial for understanding changes in alpha diversity and specific microbial taxa after surgery. A longer time gap between surgery and sample collection may influence the observed reduction in alpha diversity, as microbial populations may shift over time, potentially altering the composition of samples collected later. Additionally, this dataset is limited to patients in active disease. Patients in remission are often known to relapse and have a different microbiome from patients in active disease (39, 40). Accounting for this subset of patients could provide more insight into the differences between patients in each group, and predictors for either patients in active disease or remission.

The dataset used in the study contained information from various time points. However, we focused our data analysis on a single time point. Tracking changes over time post-surgery through a longitudinal analysis could provide deeper insights into the underlying causes driving the patterns observed. This would allow us to monitor whether the decrease in microbial diversity observed in Crohn's Disease patients following surgery is permanent or temporary. Simultaneously, we could measure the impact of different postoperative treatments on the gut microbiome diversity of patients as Crohn's Disease is managed long-term.

The inflammation score in this study was set to calprotectin levels exceeding 150 µg/g, which was consistent with the methodology used in the original study we obtained our dataset from (7). However, it is worth noting that there is no consensus in the literature regarding the optimal cutoff value for fecal calprotectin as an inflammatory marker. Currently, various studies have used different cutoff values, leading to inconsistencies across research findings (12). It would be beneficial for researchers to establish a standardized cutoff value through consensus within the scientific community to ensure greater consistency and comparability across studies, facilitating more reliable interpretations of fecal calprotectin as an inflammatory marker. Furthermore, current studies are not in agreement regarding the sensitivity and reliability of calprotectin as a biomarker to replace endoscopic and histological analyses (12). The use of certain drugs, such as nonsteroidal anti-inflammatory drug (NSAIDs) can alter fecal calprotectin levels, reducing the reliability of this biomarker (12). Beyond calprotectin, other markers like C-reactive protein (CRP), lactoferrin, and serum amyloid A are also valuable in assessing gut inflammation (44). These markers could provide a more comprehensive view of inflammatory status, particularly when used together with fecal calprotectin which could derive a multidimensional understanding of the inflammatory responses.

The dataset used in our study was limited to fecal samples collected from Caucasian participants in Sweden, and did not include information on habits or diet. The lack of demographic diversity may inhibit our ability to fully understand how these factors may influence the outcomes of our study, and potentially restrict the generalizability of our findings to broader populations. Furthermore, we must acknowledge the potential impact of confounding variables that were not controlled for in our analysis. Previous literature has shown that factors such as diet and antibiotics use may impact the gut microbiome in pediatric Crohn's Disease patients (45). Therefore, variables such as diet, antibiotic use, and other comorbidities of study participants may introduce bias and confound our interpretations and conclusions on the association between surgical intervention and bacterial diversity.

Conclusions In this study, we explored how surgical resection and disease severity affect the gut microbiome in Crohn's Disease patients. Additionally, we investigated how inflammation may modify the impact of these factors on gut microbial diversity. We hypothesized that microbial diversity would decrease in following surgery in patients with inflammation. Our findings suggest that microbial diversity is reduced significantly in Crohn's Disease patients that have undergone surgery without inflammation. Conversely, patients with existing inflammation do not exhibit significant reduction in microbial diversity after surgery. This is supported by a significant change in our alpha diversity analysis and our core microbiome analysis, indicating a reduction of microbial diversity within patients with inflammation and surgical intervention. Our analyses revealed that, although Crohn's Disease patients with worsening disease severity generally exhibited a decrease in gut microbiome diversity, as measured by both Chao1 and Shannon indices, this pattern was not observed in patients experiencing active inflammation. This suggests that inflammation may be a confounding factor, potentially obscuring the link between disease severity and gut microbiota diversity in Crohn's Disease. The findings in our paper support our hypothesis. Our discoveries offer deeper understanding into the interplay between specific gut microbiota species and inflammation in Crohn's Disease patients, paving the way for new longitudinal studies aimed at understanding how specific indicator species may be impacted by inflammation.

Future Directions Our findings provide a foundation for future studies to explore related areas of interest in Crohn's Disease severity in relation to inflammation and surgery status.

Firstly, comparing calprotectin's accuracy with other inflammatory markers like CRP can bring to light greater details regarding its role as a biomarker in Crohn's Disease. Collecting microbiome data and inflammatory markers both before and after surgery can reveal how the initial gut microbiome composition may influence disease course and response to surgery. Moreover, our analysis of indicator species showed that patients who underwent surgical resection lack important anti-inflammatory bacterial groups. Further exploration to pinpoint the specific bacterial communities linked to inflammation and their reaction to surgery could open avenues for precise interventions. Establishing a causal relationship between the microbiome under scrutiny and Crohn's Disease could prompt deeper investigation into probiotic treatments or fecal transplants involving the absent microbiota groups as potential therapeutic alternatives. These therapeutic advancements can also contribute to the development of personalized medicine approaches by considering individual variations in microbiome and inflammatory marker profiles. Finally, investigating reduction in short-chain fatty acid (SCFA) production would provide valuable insights into the disease process.

ACKNOWLEDGEMENTS

We would like to express our sincere appreciation to Dr. Evelyn Sun, Avril Metcalfe-Roach, and the rest of the MICB 475 teaching team for their invaluable support and guidance throughout our project. Additionally, we would like to thank the University of British Columbia's Department of Microbiology & Immunology for their funding support and resources. We also extend our gratitude to Halfvarson et al. for providing the dataset used in our study. We would also like to thank two anonymous reviewers for constructive feedback on this manuscript.

CONTRIBUTIONS

All authors formulated the project's initial concept and proposal equally. MB, AF, and JF completed the QIIME2 processing, diversity, indicator species analyses. RG and SZ completed the core microbiome analysis. For the manuscript, MB, JF, and SZ contributed to the abstract, results, and figures. MB, AF, and JF contributed to the methods, and AF, RG, and SZ contributed to the discussion.

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