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YEAR IN MICROBIOTA: IBD CLINICAL STUDIES

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Abstract – The current article is a review of the most important, accessible and relevant literature published between April 2022 and March 2023 on the gut microbiota and inflammatory bowel disease (IBD) based on human clinical studies. The major areas of publication during this period were human studies and animal models. There were over 55 relevant articles published in the reporting period.

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HUMAN CLINICAL STUDIES

Microbial Biomarkers

The gut microbiota in IBD is a highly heterogenous community, varying across disease severity, phenotype, geographical cohorts, and individual patients. In the past year, multiple studies were conducted to further understanding of microbial changes in IBD, which yielded the identification of both positive and negative bacterial markers. Salimi et al[1] and Xu et al[2] looked at stool microbial profiles of IBD patients in comparison to IBD-remission and healthy controls, respectively. IBD patients in both studies had decreased abundance of Firmicutes and Bacteroidetes phyla, and increased *Escherichia coli, Klebsiella pneumoniae,* and gamma-Proteobacteria, consistent with prior studies. In contrast, Salimi et al[1] and Xu et al[2] had conflicting findings in that the prior study found increased Actinobacteria in the IBD group, conflicting with the latter study of decreased abundance. Additionally, Salimi et al[1] also found enhanced Actinobacteria in the IBD-remission group. Despite some conflicting findings, consensus from these studies suggest a decrease in Firmicutes and increase in gamma-Proteobacteria could be used as indicators of IBD disease activity. Xu et al[2] found 135 differentially expressed metabolites in IBD patients of which 17 were part of discriminate pathways of microbial and metabolic interactions, suggesting potential use in differentiating IBD and healthy patients.

Buisson et al[3] identified the role of Adherent-invasive *Escherichia coli* (AIEC) in ileal CD from a post-operative recurrence (POR) model. CD patients with a history of AIEC had a higher risk of endoscopic POR, with AIEC detected at six months post-surgery associated with a higher

rate of ileal lesions. AIEC colonisation was associated with a specific microbial signature within tissue biopsies which was characterised by reduced *Lactobacillales, Burkholderiales*, and *Oscillospirale*s, and increased *Lachnospirales*, particularly *Ruminococcus gnavus* and *Ruminococcus torques*. The authors explained that biofilm formation enhanced AIEC adherence to intestinal cells, while manipulation of host immune response were potential mechanisms that enabled AIEC invasion and persistence in disease recurrence.

Shin et al[4] found that compositional changes in faecal microbiota was associated with clinical phenotypes and prognosis of IBD in Korean patients. As reported in previous studies, α-diversity of faecal bacteria were significantly lower in IBD patients compared to healthy controls. Microbial signatures also differed between CD and UC, indicating the potential in differentiating IBD phenotypes based on microbial profiles. In UC, α-diversity decreased as severity and extent of disease increased, and microbial communities were significantly different between patients with proctitis and left-sided or extensive colitis. The authors identified microbial biomarkers for disease severity and extent in UC, and active disease and ileocolonic involvement in CD. Moreover, *Lachnospiraceae* and *R. gnavus* were favourable prognostic markers for CD.

Buffet-Bataillon et al[5] identified that specific microbial profiles are associated with symptom severity, and patient heterogeneity is predictive of clinical evolution and disease relapse for CD. Alterations in certain microbial taxa, including a decrease in SCFA-producing bacteria (e.g., *Roseburia, Eubacterium, Subdoligranumum, Ruminococcus*) and an increase in pro-inflammatory bacterial species (e.g., *Proteus* and *Finegoldia*), can influence metabolic pathways and lead to disease exacerbation. Exacerbation was linked to subsequent loss of SCFA-producing bacteria including *Ezakiella, Anaerococcus, Megasphaera, Anaeroglobus* and *Fenollaria* and an increase in pro-inflammatory Proteobacterial species including *Klebsiella, Pseudomonas, Salmonella, Acinetobacter* and *Hafnia* and Firmicutes members including *Staphylococcus, Enterococcus*, and *Streptococcus*.

IBD patients in remission still have gut microbial dysbiosis including lower α-diversity compared to healthy controls, with b-diversity indices also reflecting dissimilarities between disease conditions and subtypes. Pisani et al[6] found potential components of the faecal microbiota that can sustain inflammation and induce relapse during remission. Flavonoid-degrading bacteria, such as *Flavonifractor plautii* and *Eggerthella lenta*, were significantly associated with IBD, whilst *Enterobacteriaceae* were shown to act as drivers for residual inflammation, even in low disease activity.

Abdel-Rahman and Morgan[7] presents a systematic meta-analysis demonstrating how different variables (e.g., disease severity and sample type) can influence results and reproducibility in IBD microbiome studies. They found that both CD and UC were associated with reduced α-diversity scores, and disease severity contributed to variation in b-diversity in most studies. Fusobacterium abundance was most consistently associated with CD, and Enterococcus was consistently associated with both CD and UC. However, disease-associated genera were inconsistent among studies, with variation in sample type being a significant contributor to inconsistency as stool studies showed lower heterogeneity than biopsy studies.

Zhang et al[8] presents a meta-analysis and systematic review showing a statistically significant association between prior appendectomy and an increased risk of developing CD. The authors revealed that prior appendectomy is associated with higher likelihood of complications, disease relapse, and the need for surgical intervention. It is possible that prior appendectomy disrupts the gut microbiota or alters immune responses, but further research is needed to establish a causal relationship.

Touch et al[9] found that *Faecalibacterium prausnitzii,* a dominant bacterium of the Clostridium IV group in human gut, induces the IL-10–producing double-positive CD8α (DP8α) Tregs. To understand the role of DP8α Tregs in the control of IBD, immunodeficient mice were administered DR*0401-restricted DP8α Tregs in combination with *F. prausnitzii* before induced colitis with DSS. Results showed that the combination of DP8α Tregs and *F. prausnitzii* attenuated colitis severity, including the decrease of body weight loss and DAI, lower histological score, while no such protective effect was observed when the mice were treated with DP8α Tregs or *F. prausnitzii* alone. Then based on a cohort of 250 IBD patients and 73 healthy controls, they found that the abundance of both DP8α Tregs and *F. prausnitzii* was significantly reduced in IBD patients. In the patients, low DP8α cells abundance

correlated with parameters of disease activity, such as flare and elevated CRP (>5). Their data led the authors to suggest that DP8α Treg may be an indicator of IBD and a potential therapeutic target.

Analysis of bacterial extracellular vesicles (EVs) is becoming an important consideration in IBD biomarker identification. These microbe-derived EVs are secreted by bacteria for communication between bacteria and host cells and regulate signalling and inflammatory pathways. Heo et al[10] aimed to evaluate the potential of gut microbe-derived extracellular vesicles (EVs) to differentiate between IBD and controls and also to predict disease relapse. Significant differences in diversity indices in microbe-derived EVs were determined and were better at differentiating between IBD patients and controls than stool microbiome analysis. However, microbe-derived EV profiles were equivocal in predicting relapses, with faecal calprotectin identified as the only positive risk factor.

FAECAL MICROBIOTA TRANSPLANTATION (FMT) STUDIES

Huang et al[11] compared the safety and efficacy of FMT with glucocorticoid treatment in patients with mild-moderate UC in a single-centre prospective cohort study. Patients were treated with either FMT (n=62; 150 mL FMT administered each time in 3 consecutive days) or glucocorticoids (GCs; n=60; oral prednisone 0.8-1 mg/kg/day). After 2 weeks, the prednisone dose tapered by 5 mg/week. 34 patients in the FMT group (54.8%) and 29 in the GCs group (48.3%) reached the primary outcome of clinical and endoscopic remission at 12 weeks. The total Mayo score decreased significantly in both groups with no significance between the groups at week 12. However, there was a significant difference in the incidence of adverse events with 36 participants in the GCs group (60%) observing adverse events, compared to 14 in the FMT group (22.6%). Therefore, the researchers found that FMT therapy was as effective as GCs to induce remission in active mild-moderate UC but with fewer adverse events. Patients in the FMT group were stratified into responders (RE) or non-responders (NR) and serum cytokine levels investigated. TNF-α and IL-6 levels decreased significantly in RE, while IL-10 decreased significantly in NR. Therefore, FMT may have an anti-inflammatory effect to induce remission in UC, resulting in the alteration of gut microbiota related cytokine expression.

A randomised trial examining the effects of antibiotic pre-treatment and comparing two methods of maintenance dose delivery was conducted by Smith et al[12] consisting of 22 patients with mild-moderate UC. Patients were randomised into arms receiving antibiotic pre-treatment (ABX+, n=11) or not (ABX-; n=11) and maintenance doses via either enema or capsules. Patients in the ABX+ group received neomycin, vancomycin and metronidazole 500 mg twice daily for 5 days followed by a one day wash out period. Increased clinical remission was seen in patients receiving antibiotic treatment (6/9; 67%) compared to non-antibiotics (2/11; 18%). If the patients Mayo score decreased by 3 or more points, they were classified as responders. 10 of 22 patients were responders, with no statistical association with pre-treatment, maintenance method, or donor, tested individually by logistic regression. Smith et al[12] found that FMT strongly affects the taxonomic composition of the patients' gut microbiomes with patients faecal communities were more similar to their prospective donor that their original composition. There was a trend towards increased remission rates after FMT in the ABX+ group, but this did not reach statistical significance. It is possible that antibiotic pre-treatment could result in a greater transfer of microbial functions as it was found that transmission of donor microbiota was significantly increased after FMT in the ABX+ group. Consistent with previous findings on FMT treatment of *C. difficile* infection, maintenance does through capsules versus enemas lead to similar strain transmission and remission rates. Despite the lack of a control or placebo group, it has shown a potential increase in the UC remission and transfer of donor microbiota during FMT in patients receiving antibiotic pre-treatment.

Gholam-Mostafaei et al[13] investigated alterations to intestinal microbiota following FMT in 7 IBD patients with *Clostridioides difficile* infection (CDI). Microbial analysis was performed by RT-qPCR assay targeting the 16S rRNA gene. All 7 patients recruited in this study had symptoms of CDI resolved within 2 months post-FMT and the PCR results demonstrated complete resolution of CDI in all patients. In pre-FMT samples, Firmicutes was the most abundant phyla, whist Bacteroidetes had the highest abundance in post-FMT and donors.

Post-FMT faecal microbiota analysis at the phylum level demonstrated significant differences in the composition of Firmicutes, Bacteroidetes and Spirochaetes phyla, compared to the pre-FMT microbial profile. At the class level, Delta-Proteobacteria was the most abundant one in pre-FMT samples whereas Beta-Proteobacteria was the least abundant in the pre-FMT samples but was most abundant in the donors. The Firmicutes/Bacteroidetes ratio significantly reduced in the post-FMT group, becoming closer to the donors' microbial composition over time. The microbiota profile of FMT recipients became more similar to donor samples. Although the study only had a small sample size and was limited to RT-qPCR analysis it demonstrated that FMT is an effective and safe therapeutic strategy to restore the native microbial composition in patients with CDI and IBD.

The impact of diet in addition to FMT therapy was explored in 2 studies involving patients with UC. In a single, blinded randomised controlled trial in adults with active UC, Shabat et al[14] evaluated whether an ulcerative colitis exclusion diet (UCED) in addition to FMT could increase UC remission rates. Included 3 groups (1) ate a free diet and received standard FMT; (2) received FMT from donors with dietary pre-conditioning of the donor combined with UCED after transplantation and (3) received UCED only. FMT was administered by colonoscopy at Day and rectal enemas on day 2 and 14. Eight donors and 62 patients were enrolled in the study. Intention to treat response and remission rates were 35.3% and 11.8% for group 1, 42.1% and 21.1% for group 2 and 60% and 40% for group 3. Endoscopic remission ay week 8 was highest for patients in group 3, 26.6% achieved and lowest in group 1 (11.7%) Mayo endoscopic score of 0 was achieved in 20% patients from group 3 and no patients receiving FMT. In this study, FMT was largely unsuccessful in producing patient clinical remission, whilst UCED alone had higher remission rates without FMT. The authors speculate that diet alone may have succeeded better than FMT with diet as FMT during inflammation may have destabilised the microbiome further in patients who flared or did not respond to FMT. However, the trial was stopped by a safety monitoring board for futility, which could be due to the inclusion of only inflamed patients with more than half failing steroids or biologics at enrolment.

In contrast, Kedia et al[15] found that FMT in combination with an anti-inflammatory diet was more effective than optimised standard medical therapy at stimulating remission in mild-moderate UC. In this single-centre, prospective, open-labelled RCT, patients were randomised into FMT and anti-inflammatory diet (FMT-AID; n=35) or optimised standard medical therapy (SMT; n=31) arms. Patients in the FMT-AID arm received a diet chart and were counselled to adhere to the diet protocol. The AID avoided gluten-based grains, dairy products, processed and red meat, food additives and refined sugars and increased intake of fresh fruit and vegetables, fermented foods, AhR ligand-rich vegetables and polyphenols. FMT from rural donors was administered by colonoscopy weekly for 7 weeks. In the FMT-AID arm, 23/35 (65.7%) patients achieved clinical response and 21/35 (60%) achieved clinical remission, which was significantly higher than the SMT arm [11/31 (35.5%) and 10/31 (32.2%), respectively]. The endoscopic response was also significantly higher in the FMT-AID arm compared to SMT [17/33 (51.5%) *vs.* 4/23 (17.4%)]. Additionally, 23 clinical responders in the FMT-AID arm and 11 in SMT entered the maintenance phase of steroid-free clinical remission at 48 weeks, suggesting that anti-inflammatory diet could maintain the FMT-AID-induced remission.

In the study above by Kedia et al[15] FMT was specifically obtained from rural donors based their previous study that rural healthy donors have a superior gut microbiome signature. FMT donor selection is crucial for FMT efficacy. Haifer et al[16] previously found that FMT from donor 1 had 100% efficacy whereas donor 2 only had 36% efficacy. Therefore, they characterised the differences in gut microbiota of these donors to improve FMT donor selection. Faecal samples from 2 donors were collected over 44 (donor 1) and 70 (donor 2) weeks. Donor 1 showed robust stability in species richness over time whereas Donor 2 had larger fluctuations. Donor 2 had significantly greater species richness, however donor 1 had significantly greater diversity at the phylum level. The relative abundance of Prevotella copri in donor 2 appeared to contribute to the lack of stability. As donor 1 had a significantly higher and stable species evenness it was hypothesised that species evenness could be a marker of higher intracommunity stability and species availability on transplant inducing patient outcome. This hypothesis was validated using donor batches from the FOCUS clinical trial

which showed >75% of patients reaching primary endpoint had significantly higher species evenness and less dispersion than batches that resulted in <25%. The authors found that donor microbiota stability and species evenness are highly relevant to efficacy of FMT transfer and patient outcomes and proposed a novel framework for donor selection.

Only one study considered gut fungal communities in FMT. Chen et al[17] conducted a clinical trial to determine the association between the gut fungal community and capsulised FMT in patients with UC. Patients with active UC (n=22) received capsulised FMT 3 times/week from at least 2 randomly assigned donors. Using shotgun metagenomic sequencing, it was found that overall fungal community in patients with UC was significantly clustered from the donor samples in beta-diversity analysed using PCA.

GEOGRAPHICAL VARIANCES IN IBD MICROBIOME

Pilot studies from Malta Rausch et al[18], Southwest China Wang et al[19], and Saudi Arabia Al-Amrah et al[20], sought to characterise IBD intestinal microbiota profiles in their respective geographic regions. In Buenos Aires, Rosso et al[21] also looked at metabolomics and epigenetic markers in their cohort. These studies confirmed previous findings of dysbiosis, while pointing out relevant markers of elevated Proteobacteria and Fusobacterium in Southwest China, and negative markers of *Paraprevotellaceae*, *Muribaculaceae* families of Bacteroidetes phylum, and *Leuconostocaceae* family of Firmicutes phylum in the Saudi Arabian cohort. In the Mediterranean, Rausch et al[18] found that microbial differences in early disease were not significant as a marker for disease severity, while also suggesting geographical and population factors as explanations for the variations in dysbiosis as compared to previous studies.

To better understand geographical variances in IBD dysbiosis, Mayorga et al[22] compared microbiome data from Spain, China, and the United States. They found that on a global level, geography and phenotype were the main covariates in microbiome variations. In UC especially, geography was the most prominent factor, while disease location was the more important factor in CD. Across various geographies, alpha-diversity differed independent of health status, while CD harboured similar microbial taxonomic profiles across regions. This study suggests that geographic location, environmental factors, and disease activity are important factors in impacting microbial changes and must be taken into consideration for valid and reproducible profiles and markers.

Paediatric IBD

Olbjørn, Småstuen and Moen[23] analysed the gut microbiota in paediatric IBD and found that the faecal abundance of several bacterial species was statistically reduced compared to both symptomatic patients and healthy controls. CD patients had reduced *Bifidobacterium* spp. than UC patients, and those with stricturing and/or penetrating phenotypes had decreased abundance of *Christensinella minuta*, *Clostridium scindens*, *Eubacterium eligens*, and *Roseburia hominis*, and increased abundance of *E. coli*, compared to inflammatory phenotype. Furthermore, paediatric patients that required biologic therapy had lower abundance of butyrate-producing bacteria. The authors introduced Diagnostic, Phenotype, and Prognostic Indexes that have good discrimination properties, which can aid IBD diagnosis and identify the need of biologic therapy in paediatric patients.

Hellmann et al[24] assessed gut microbiota signatures and disease phenotype in a paediatric IBD cohort while controlling for mucosal inflammation by faecal calprotectin measurements. They found that microbial shifts may underlie and drive symptoms regardless of mucosal inflammation. Correlations seen in previous studies were confirmed, including rectal bleeding and stool frequency associating with increased *Klebsiella* and reduced *Bacteroides* species. When controlling for mucosal inflammation, UC patients with lower calprotectin had reduced *Klebsiella,* and both CD and UC patients displayed less longitudinal microbial community stability. Breton et al[25] defined the microbial signature specific for perianal fistulising CD in paediatric patients, in which the fistula-associated microbiome profiles displayed increased alpha diversity and altered abundance of multiple taxa, particularly the Proteobacteria phylum,

compared to rectal- and faecal-associated microbiomes. There was also reduced butyrogenic potential in the mucosal-associated microbiome of perianal CD than non-perianal phenotype and healthy individuals. The authors also identified genes resistant to ciprofloxacin and metronidazole. Overall, this can guide development of novel microbiome-based therapies for this CD phenotype.

Schmidt et al[26] characterised the composition of mucosally-adherent duodenal microbiome in paediatric CD patients, which was characterised by increased *Bacteroidales*, specifically *Prevotellaceae*, in the actively inflamed duodenum. This was significantly different to those without CD, which was characterised by increased *Pseudomonodales* and *Spirochetes*. The authors also found that the paediatric duodenal microbiome was significantly distinct from the terminal ileum, but there were no statistically significant correlations between bacterial abundance and age, sex, medication use, or villous length.

Pharmacological IBD Therapies and Microbiota Changes

We start this section on pharmacological IBD therapies with a systematic review and meta-analysis of studies that reported longitudinal microbiota analysis using next-generation sequencing or high-throughput sequencing of faecal and mucosal samples from IBD patients commencing treatment, conducted by Mah et al[27]. The analysis suggests that IBD treatments, including non-biological, biological, and nutritional therapies, alter gut microbiota profiles, and there is growing evidence that the gut microbiota reciprocally influences therapeutic efficacy. The meta-analysis of alpha-diversity changes following infliximab treatment showed a significant increase in alpha-diversity, indicating a positive effect on the gut microbiota. Critically, the authors state the need for more longitudinal studies of IBD cohorts that are adequately powered to establish links between these two facets further.

Park et al[28] investigated whether microbiome changes at multiple sites (from stool, saliva, serum and urine) can predict the effectiveness of anti-tumour necrosis factor-α (TNF-α) treatment in patients with IBD. The study collected samples from 19 IBD patients before and after anti-TNF-α treatment and from 19 healthy subjects. Microbiota analysis was performed using extracellular vesicles and next-generation sequencing. Using NGS analysis, they found that the stool was the only sample type where α-diversity differed significantly between the IBD and control groups before and after treatment. Responders to anti-TNF-α treatment had significantly higher levels of Firmicutes, Clostridia, and other microbial changes in stool than non-responders. The study suggested that stool microbiome changes may be a valuable predictor of the effectiveness of anti-TNF-α treatment in IBD patients.

Along the same vein but focusing on the IBD therapy 5-aminosalicylic acid (5-ASA), Mehta et al[29] demonstrated the gut microbiota's capability to metabolise the drug into inactive metabolites and thereby reduce clinical efficacy. In their analysis of metagenomics data sourced from the IBDMDB cohort, evidence was presented to suggest that the key culprits of 5-ASA degradation were *Clostridium scindens* and *Eggerthella lenta*. This was further narrowed down to specific bacterial enzymes which identified an association between three microbial thiolases and one acyl-CoA-acyltransferase.

A study by Lv et al[30] reported that a combined dose of *Lactobacillus plantarum* alongside tacrolimus in a DSS mouse colitis model enhanced the therapeutic effect compared to tacrolimus alone. Mice given the combined treatment had prolonged survival time, greater suppression of body weight loss and colonic mucosal inflammation relief. Additionally, the immune and inflammation-related signalling pathways interferon (IFN)-γ, IFN-α and IL-2 signal transducer and activator of transcription (STAT)5 were downregulated.

Wu et al[31] assessed the effect of deferasirox (an oral iron chelator drug) on severity, ferroptosis (an iron-dependent form of apoptosis), and gut microbiota using the DSS murine model of colitis. The results showed that deferasirox treatment significantly alleviated colitis severity and inhibited ferroptosis in the colon. The study also identified specific gut bacteria that were modulated by deferasirox treatment. *Lachnospiraceae*, *Prevotellaceae*, *Ordoribacter* and *Blautia* were increased, while *Escherichia Shigella* and *Streptococcus* were significantly decreased. The findings suggest that deferasirox has the potential as a therapeutic agent for UC by inhibiting ferroptosis and improving the gut microbiota.

Eckenberger et al[32] examined microbiota variance in IBD to determine the degree to which medication might account for compositional differences between disease subtypes and geographic location. This study builds on their previous findings using the same cohort of CD and UC patients from Cork, Ireland and Manitoba, Canada, where they demonstrated that geographical location had a major influence on gut microbiota variance. Indeed, the current study aimed to disentangle the effect of differences in treatment from the apparent geographical influence. Eckenburger et al[32] found that treatment explained more microbiota variance (3.5%) than all other factors combined (2.4%), and 40 of 78 tested medications significantly correlated with at least one gut microbial taxon. This work, alongside the preceding studies on IBD drugs, contributes to the body of evidence of a bidirectional relationship between IBD therapeutics and the gut microbiota. Methods of patient profiling, including at the microbial level, may provide better matches to therapies that will provide the greatest efficacy.

Reinisch et al[33] reported on a phase 1b study evaluating the safety, pharmacokinetics, and pharmacodynamics of sibofimloc, a novel FimH blocker, in patients with active Crohn's disease (CD). FimH is an adhesin expressed by invasive *E. coli* which binds to epithelial glycoproteins and stimulates pro-inflammatory cytokine release. The study involved eight patients with active ileal or ileocolonic CD who received a single oral dose of 3000 mg sibofimloc followed by 1500 mg twice daily for 13 days or 1500 mg sibofimloc twice daily for 13 days. They showed that systemic sibofimloc exposure was low, and the drug was well-tolerated with no serious adverse events reported. In addition, pro-inflammatory markers, including IL-1β, IL-6, IL-8, and TNF-α, were decreased in patient stool after the study period.

Janus kinase (JAK) inhibitors have come under scrutiny due to the risk of adverse effects despite their demonstrable efficacy and FDA approval (two products) for UC treatment, with several other inhibitors in late-stage clinical trials. Yadav et al[34] proposed a strategy to circumvent the adverse side effects of the available gastric release medication by explicitly targeting the ileocolonic delivery of JAK inhibitor tofacitinib in mice. The method for targeted delivery involved encasing tofacitinib tablets with a commercial coating designed to resist degradation in the upper GI tract and selectively break down in the human colon. This was compared with a liquid drug formation delivered by oral gavage. After confirming tofacitinib stability in the presence of mice caecal slurry *ex-vivo*, the study demonstrated that ileocolonic-targeted delivery of tofacitinib led to increased tissue exposure and reduced systemic exposure compared to untargeted formulations.

Vezza et al[35] assessed the broad-spectrum antibiotic minocycline as a therapy for managing visceral pain in a DSS murine model of colitis. Their findings demonstrated that minocycline treatment reduced histological features of intestinal inflammation and expression of inflammatory markers, including IL-1β and TNF-α. Vezza et al[35] also observed restitution of gut microbiota profiles induced by the DSS: including restoration of *Bacteroides*, *Romboustia*, *Prevotellaceae* and *Faecalibaculum*, after treatment with minocycline. A reduction in pain, assessed by infra-red video footage of mice's facial expression, coincided with gut dysbiosis reversal and inflammatory marker reduction in the minocycline treatment group.

We close this section with an innovative engineered procyanidin (Pc) and free iron (Fe) nanozyme (Pc-Fe) and assessment of its therapeutic potential in a DSS model of mouse colitis. Chang et al[36] engineered a nanoparticle to address the stability and solubility limitations of the otherwise promising antioxidant Pc. Previous research had demonstrated its ability to eliminate reactive oxygen species (ROS) in conjunction with the excess accumulation of ROS in IBD development, presenting a case for the therapeutic potential of Pc. The results demonstrated that Pc-Fe nanoparticles significantly alleviated colitis severity by scavenging ROS and altering the gut microbiota. The study also identified specific gut bacteria that were altered by Pc-Fe nanoparticles. Histological and biochemical assessment of mice's liver and kidney revealed no apparent adverse effects from Pc-Fe treatment.

NON-BACTERIAL AND MULTI-OMICS STUDIES OF THE GUT MICROBIOTA

Imai et al[37] characterised the human gut virome in a Japanese IBD cohort, and identified viral signatures associated with CD, in which the overall virome structure was significantly different to healthy individuals. Caudovirales (e.g., CrAssphage and *Staphylococcus* virus) are dominant

in both CD and HC, but *Lactococcus, Enterococcus*, and *Lactobacillus* phages were only found in CD, while *Xanthomonas* and *Escherichia* phage were only found in HC. Significant interactions between viruses and bacteria suggested the potential role of altered gut virome composition in bacterial dysbiosis and CD pathogenesis. Other virome information was reported by Stockdale et al[38] who compared unamplified gut viromes to 16S rRNA gene analysis and found that a- and b-diversity metrics of unamplified gut viromes were less efficient at diffexrentiating HC from IBD.

Eukaryotic microorganisms are often overlooked in the gut microbiota. Guzzo et al[39] sought to characterise fungal and protozoal changes in IBD in a cohort of 355 patients. They found a higher prevalence of fungi, especially *Saccharomyces cerevisiae,* and lower prevalence of protozoa, especially *Blastocystis*, in IBD patients. Disease severity, body mass index, and age were associated with the abundance of these two genera. The results demonstrated that some patients had eukaryotic species that remained stable over time. Further research into eukaryotic profiles in IBD could aim to guide diagnostic and therapeutic assessment.

Shome et al[40] conducted a case-control study to identify anti-microbial antibody signatures in IBD patients. They found that antibodies against *Bacteroidetes vulgatus* and *Streptococcus pneumonia* antigens were more prevalent in CD than HC, while antibodies against *Streptococcus pyogenes* antigens were more prevalent in HC than UC. Whilst antibody signatures can increase our understanding of the role of source microorganisms in IBD pathogenesis and benefit clinical management, more studies are required.

MULTI-OMICS STUDIES OF THE GUT MICROBIOTA

Vila et al[41] explored the IBD faecal metabolome and identified more than 300 differentially abundant metabolites, indicating potential associations between metabolite profiles and IBD. The findings showed that the faecal metabolome is significantly distinct between IBD patients and HC, with a major discriminator being the ratio between sphingolipid and L-urobilin. The authors found changes in the bile acid pool is associated with dysbiotic communities, and there are strong associations between faecal metabolomes and the gut microbiota. Variations in faecal metabolome can be caused by phenotype, treatment, microbial composition, and dietary patterns, and further research is needed for clinical use as non-invasive biomarkers or therapeutic opportunities for IBD.

Bile acid (BA) metabolites, tryptophan metabolites, and short-chain fatty acids (SFCA) play important roles in inflammatory regulation in IBD. BAs in particular function by activating receptors to regulate energy (glucose and lipid) metabolism and are derived from cholesterol in the liver. Ju et al[42] looked specifically at the BA of deoxycholic acid (DCA) and found that it was significantly decreased in patients with IBD and associated with a decreased abundance of commensal flora. Additionally, the impact of ileocolectomy-induced BA perturbations on the gut microbiota was explored by Battat et al[42]. They found that elevated primary BAs were associated with reduced microbial diversity, ileitis, *F. prausnitzii* abundance and certain enzymatic abundances.

Gonzalez et al[44] defined location-specific signatures of CD using mass spectrometry (i.e., metabolomics or metaproteomics) with combined multi-omics methods which showed that significant differences existed between colonic and ileal CD in stool. Colonic CD had severity-related association with *Bacteroides vulgatus*, and similar to UC, was strongly associated with neutrophil-related proteins, highlighting the integral role of host inflammation and proteolytic activity in IBD. Ileal CD demonstrated alterations in primary and secondary bile acid levels, and taxa with noted sensitivities, including *F. prausnitzii*, or affinities for environments rich in bile acid, such as Gammaproteobacteria and *Blautia* species. Overall, the authors identified microbial and molecular differences between CD locations, and proposed location-specific biomarkers to monitor disease severity in clinical practice.

Sudhakar et al[45] examined microbe-host interactions in CD patients by integrating community-wide microbial profiles with host transcriptomic data to understand potential mechanisms of microbial proteins on host gene expression. They found that intestinal inflammation was associated with gut microbial alterations since bacterial proteins of enriched abundance or transcriptional activity activated inflammatory responses by eliciting post-translational modifica-

tions on host receptors and derepressing pro-inflammatory cytokines. The authors also found site-specific mechanisms and highlighted proteins of topological and functional relevance that can be targeted for potential treatment strategies in IBD.

Ma et al[46] develop a novel statistical framework, MMUPHin, for normalization, statistical meta-analysis, and population structure discovery using microbial taxonomic and functional profiles, to enable microbial community meta-analyses. Combining sequence homology, secondary-structure-based functional annotations, phylogenetic binning, ecological distribution and environmental or phenotypic statistics. Zhang et al[47] developed a workflow, named MetaWIBELE, to identify novel bioactive elements that are linked to IBD in the microbiome.

IBD, CO-MORBIDITIES, MICROBIOTA CHANGES AND CLOSTRIDIOIDES DIFFICILE

Defining intestinal microbiota signatures of IBD patients with concomitant *Clostridioides difficile* infection (CDI) remains an under investigated field despite the long association of CDI with poorer disease outcomes (larger medication doses, longer hospital stays and higher colectomy rates). Yu et al[48] assessed gut microbial profiles to identify potential biomarkers in IBD-CDI patients. The findings identified biomarkers including *Ruminococcus gnavus*, *Clostridium innocuum*, and *Enterococcus faecium*, with distinguishing fungal taxa including *Saccharomyces cerevisiae* in IBD-CDI patients. They also found a decreased prevalence of *Faecalibacterium* numbers in patients with IBD-CDI compared to IBD patients without CDI. *Faecalibacterium* induce anti-inflammatory effects in the gut via regulation of butyrate-mediated pathways. Increased proportion of *Enterococcus*, especially *Enterococcus faecium* was another of the differential biomarkers in IBD-CDI, with *E. faecium* involved in peptidoglycan metabolism pathways that can block targets of antimicrobial agents. Further research into positive and negative markers in this subset of IBD patients could aid in pre-therapeutic assessments and management.

Faecal microbial transplantation (FMT) can also play a role in restoring microbial diversity in patients with IBD and recurrent *CDI*. FMT has been shown to be effective in treating CDIs, but its use in concurrent CDIs and IBD has not been studied. Ramos et al[49] sought to explore this question further and established that FMT restores alpha-diversity in IBD-CDI patients. In addition, certain markers with immunomodulatory activities like secondary bile acid levels which are reduced in IBD-CDI cohorts were restored post-FMT. This was correlated with increased metagenomic findings (baiE gene) and eight bile salt hydrolase phylotypes, which the researchers proposed could play a role in future disease monitoring and therapeutics.

IBD AND DEPRESSION

The brain-gut axis is a bi-directional communication channel that involves neuroimmune, endocrine, and inflammatory mechanisms and existing evidence suggests alterations in abundance and diversity of gut microbiota play a role in pathogenesis and pathophysiology of depression. Qin et al[50] and Thomann et al[51] found interactions present in IBD patients with depression between certain genera of bacteria and functional modules. Specifically, *Odoribacter*, *Anaerotruncus*, and *Alistipes* genera, along with the functional modules of pectin, glycosaminoglycan, and central carbohydrate metabolism, were associated with depression. More than 64 candidate interactions were associated with depression risk as well as multiple candidate genes, although the underlying mechanisms remain to be explored. The study provides evidence that microbiota-directed therapies could potentially reduce fatigue and depression in IBD and should be further explored in future research.

IBD AND OBESITY

The incidence of overweight/obesity and IBD are increasing in parallel, with > 40% of IBD patients estimated to be obese. Studies have shown conflicting findings regarding the correlations of obesity and IBD severity. Studies suggest increased complications in obesity due to hospi-

talisation rates, perianal complications, and delays in surgery[52]. However, other studies found correlations of higher BMI with better prognoses, such as reduced severity, fewer biologics, surgeries, or hospital stays[53]. Yan et al[54] (n=64) looked at IBD patients with and without overweight/obesity. They found significant decreases in serum inflammatory response-associated proteins in overweight/obese IBD patients compared to non-overweight/obese counterparts, which could be an indicator for less severe disease course of IBD. Faecal metaproteomic analysis also provided a series of positive and negative markers in UC and CD patients, which may be used as markers in assessment of IBD.

RESPONSE TO SARS-COV-2 VACCINATION

Immunosuppressive therapy in IBD such as anti-TNF medications such as infliximab, result in lowered humoral responses to vaccinations against SARS-CoV-2. A study by Alexander et al[55] on gut microbiota profiles of 43 Infliximab-treated patients showed *Bilophila* was associated with stronger serological response, postulated as acting as a vaccine adjuvant in engaging T-cell support, while *Streptococcus* was associated with poorer response. Microbial metabolites were also distinct between poor and strong humoral responders to vaccinations, in particular trimethylamine (TMA) correlated with stronger responses. TMA is a precursor to TMA N-oxide which is implicated in enhancing cancer immunotherapy. These results indicate that therapeutics targeted at modulating the gut microbiota or supplementing beneficial metabolites may ameliorate poor vaccine responses in vulnerable groups.

CONCLUSIONS

According to the last years reporting clinical studies in Microbiome, IBD research continues to expand and extend our understanding and reaffirms the intense interest in the field. Future research must focus on defining key mechanisms relevant to pathology and therapeutic responses to continue to address the critical gaps in the field.

Conflict of Interest

There is no conflict of interest to declare.

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Author's Contribution

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