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Review Article

Understanding Inflammatory Bowel Disease: An Integrative Framework of Microbiome, Metabolome, and Immunological Biomarkers

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Abstract

Inflammatory bowel disease (IBD) is characterized by chronic inflammation of the gastrointestinal tract and primarily includes ulcerative colitis and Crohn's disease. As the number of patients suffering from IBD increases, diagnosis and treatment have become pressing yet challenging tasks. A major challenge is that patients with IBD often do not exhibit characteristic symptoms, making it difficult to distinguish IBD from other intestinal abnormalities. Endoscopy is the most conventional method used to diagnose IBD; however, this technique is invasive and costly. Therefore, there is a need to develop affordable, non-invasive diagnostic methods, which underscores the importance of identifying biomarkers specific to IBD. It is now well established that the gut microbiome plays a significant role in the development of IBD, and changes in the abundance of various gut organisms have been widely studied to identify microbial signatures associated with the disease. This review discusses the current state of knowledge regarding biomarkers in IBD, with a primary focus on the gut microbiome, associated metabolic signatures, and their links with immunological biomarkers. These biomarkers will help propose an integrative model to better understand the pathophysiology of this complex disease. Such an integrated approach also offers insights into potential therapeutic targets for designing more effective treatment strategies for patients.

Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal (GI) tract, subdivided into ulcerative colitis (UC) and Crohn's disease (CD).[1] IBD is associated with a range of complications that can significantly impact quality of life and health outcomes.[2] The predominant symptoms—such as diarrhea, abdominal pain, GI bleeding, weight loss, malnutrition, and fatigue—can have substantial psychosocial consequences.[3] Other health complications include strictures, fistulas, abscesses, and an increased risk of colorectal cancer, particularly in patients with long-standing disease or extensive colonic involvement.[4] Extraintestinal manifestations, such as arthritis, skin disorders, and liver disease, further contribute to the burden of IBD, underscoring the need for comprehensive management strategies.[5]

Endoscopy remains the primary method for diagnosing IBD; however, it is invasive and time-consuming.[6] Technologies such as artificial intelligence (AI), endocytoscopy, and molecular imaging have significantly enhanced endoscopic examinations, providing more accurate and detailed insights into IBD detection.[7] AI improves risk prediction, genetic data analysis, and disease severity assessment through image analysis.[8] Endoscopic molecular imaging offers valuable insights by assessing disease severity, predicting treatment outcomes, and detecting dysplasia even in inflamed tissue.[6] While endocytoscopy provides highly detailed, real-time microscopic views of tissue during an endoscopy, with magnification up to 1,400 times,[9] these advances still highlight the need for non-invasive diagnostic methods.

In IBD, immune system dysregulation drives disease progression through inflammatory mediators that perpetuate local inflammation, alter gut permeability, and result in gut dysbiosis.[10] Employing gut microbiota and their metabolite biomarkers, which are characteristic of the disease, appears to be a promising non-invasive approach for diagnosis and monitoring. This strategy can aid in early detection and prediction of IBD, enabling timely interventions and reducing the risk of complications.[11] This highlights

Keywords: Inflammatory bowel disease; Microbial biomarkers; Metabolomic biomarkers; Immunological biomarkers; Integrated biomarker framework; Crohn's disease; Ulcerative colitis.

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the crucial, multifunctional role of various biomarkers in IBD, which can be utilized for diagnosis, treatment planning, and assessing mucosal healing in patients.[12] This review examines the complex interplay among gut dysbiosis, metabolite alterations, and immunological responses in IBD. It seeks to clarify the mechanisms through which alterations in the gut microbiome and its related metabolites contribute to IBD development, emphasizing specific microbial signatures, metabolomic changes, and immune biomarkers associated with disease activity, progression, and treatment response. Furthermore, it discusses the emerging roles of AI and machine learning (ML) in the diagnosis, management, and personalized treatment of IBD. Through this integrative analysis, the article aims to enhance our understanding of IBD's multifactorial nature and identify potential avenues for future research and clinical application.

Global burden and prevalence

Over the past few decades, the global burden of IBD has been rising, largely driven by changes in environmental, genetic, and lifestyle factors. Between 1990 and 2019, the global IBD population increased substantially, rising from 3.3 million to 4.9 million,[13] with China and the United States reporting the highest numbers of cases.[14] A 2019 study revealed that rates of IBD occurrence, deaths, and disability-adjusted life years were higher in older individuals compared to younger ones. Interestingly, men generally exhibited higher rates for these indicators than women until approximately 85 years of age, after which women had higher rates.[15] The study also found that the highest number of IBD cases occurred in the 50–54-year age group for women and the 60–64-year age group for men. Additionally, the mortality rate associated with IBD was highest among individuals aged 95 years and older.[13] Individuals with a family history of IBD, particularly CD, have a significantly increased risk of developing the condition. Siblings of CD patients are 13 to 36 times more likely to develop IBD, while those with a sibling affected by UC have a seven- to seventeen-fold higher risk. The presence of multiple affected family members further elevates this risk, especially in children. Offspring of two IBD-affected parents face the highest risk, ranging from 33% to 52%.[16] A comprehensive analysis of 491 studies demonstrated a dramatic increase in both CD and UC rates over the past century. Incidence rates for CD and UC surged from fewer than one to over nine and fourteen cases per 100,000 people, respectively, with significantly rising prevalence trends.[17] In 2021, a study in Iran analyzed the cost of illness, including medical treatments and other expenses, for IBD and found the annual cost per patient to be $1,077 for UC and $1,608 for CD. Patients over 40 years of age incurred the highest costs, with nationwide totals reaching $8.2 million for UC and $7.1 million for CD.[18] A 2022 European study involving 3,687 IBD patients across 12 countries found that disease costs varied based on factors such as disease type, activity, comorbidities, age, gender, country, and healthcare system characteristics. UC patients generally had higher costs, particularly for medication.[19]

Factors affecting IBD

The etiology of IBD involves a complex interplay among genetic, environmental, and microbial factors (Fig. 1).[20] Among the genetic factors, human leukocyte antigen (HLA) genes, particularly HLA class II molecules like HLA-DRB1-1502 and HLA-DRB1-1501, are strongly associated with UC, while HLA-DR4 is linked to CD in certain populations. These genes influence immune responses

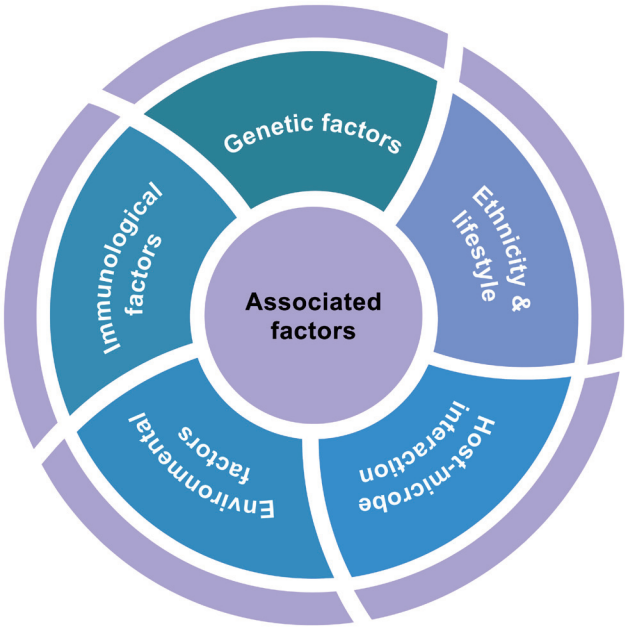

Fig. 1. Integrated view of the factors associated with IBD. IBD, inflammatory bowel disease.

by presenting antigens to T cells, playing a critical role in disease susceptibility. Ethnic variability and clinical heterogeneity further contribute to the diverse genetic associations observed in IBD. Polymorphisms in cytokine genes, such as TNFα and LTα in the MHC class III region, regulate immune system activity and are linked to both UC and CD, although findings are often inconsistent. The interleukin (IL)-1 receptor antagonist gene allele 2 is associated with increased disease severity in UC and outcomes like pouchitis, highlighting its potential role in modulating inflammation. Family linkage studies also identify genetic predispositions, suggesting linkage disequilibrium near disease susceptibility loci. Other immune-related genes, such as ICAM-1, complement C3, and T-cell receptor genes, show conflicting associations, underscoring the complexity of IBD genetics. Collectively, these findings illustrate the intricate interplay between genetic predisposition and immune dysregulation in the development of IBD.[21] Environmental factors, primarily diet, play a more significant role than genetics in shaping the microbiome; however, specific genotype–microbiome interactions remain important in IBD. For instance, the NOD2 gene variant is associated with an increased abundance of Enterobacteriaceae. A subset of microbiome taxa or functions, such as oxidative stress resistance, may be more directly linked to IBD despite environmental factors driving most microbiome variations.[22] Given this complex array of influences, it is crucial to investigate the interplay among these factors for a more detailed understanding of the causes and potential treatments. This review discusses the gut microbiome and metabolome in detail, as well as their integration with immune-related biomarkers in IBD.

Microbiome signatures in IBD

The microorganisms, including a variety of bacteria, fungi, and viruses, in the lower GI tract form an enormous and complex ecosystem.[23] Beyond changes in the gut bacteriome of IBD patients, recent studies have reported remarkable alterations in the gut my-

cobiome and virome.[23] This suggests that non-bacterial microbes, such as fungi and viruses, might also play unique and important roles in IBD pathogenesis and disease activity. Most gut microbes are beneficial and exert immunoprotective effects by regulating host immune cells. However, due to disease conditions or imbalances in the host system, alterations in the gut microbial ecosystem may occur, leading to microbial dysbiosis. Such disruptions may contribute to chronic intestinal inflammation and impaired gut barrier function, as seen in IBD. In this context, there is a shift in microbial populations, alongside inflammation or infection caused by contact between microbes and the damaged intestinal lining.[24] Microbial dysbiosis has been demonstrated in both UC and CD.[23]

Compared to healthy individuals, the structure of the gut microbiota is significantly altered in IBD across different taxonomic levels.[25] Changes in gut microbiota composition can occur early in IBD development.[26] Thus, both diversity and composition of the gut microbiota are critical factors in disease progression. IBD significantly impacts alpha and beta diversity of the gut microbiome—where alpha diversity refers to diversity within local communities (habitats), and beta diversity refers to the spatial variation in species composition between communities.[27] Studies show that patients with IBD exhibit reduced alpha diversity compared to healthy controls.[28] This reduction renders the microbiota functionally less capable and less redundant, thereby increasing vulnerability to perturbations.[29]

Gut bacteria

More than 99% of the healthy gut bacteriome consists of species from four phyla: *Firmicutes, Bacteroidetes, Proteobacteria*, and *Actinobacteria*.[23] In IBD, there is a significant reduction in beneficial bacteria such as *Faecalibacterium prausnitzii (F. prausnitzii)* and members of the *Ruminococcaceae* family,[30,31] with the exception of *Ruminococcus gnavus*,[32] members of the *Leuconostocaceae* family,[30] and *Bifidobacterium* species (Table 1).[32–68] Conversely, pathogenic bacteria such as *Campylobacter concisus,* enterotoxigenic *Bacteroides fragilis, Escherichia coli (E. coli)*, *Bacteroides fragilis*, *Fusobacterium nucleatum*, and *Mycobacterium avium* subspecies *paratuberculosis* are increased.[35]

Short-chain fatty acids (SCFAs), produced by gut microbiome members, are important for GI tract homeostasis.[69] The association between SCFAs and IBD was suggested when alterations in associated microbiota, such as *Bifidobacteria*, were observed to affect SCFA levels.[33] In UC patients, a reduction of *Bifidobacteria* in the colon was observed, which is an important SCFA producer.[34,70] SCFAs and gut microbiota in IBD influence reactive oxygen species, whose increased levels can damage the mucosal layer of the GI tract.[71] This damage may increase intestinal permeability, resulting in a leaky gut. Microbes such as *Streptococcus*, *Bifidobacterium*, and *Lactobacillus*, often administered as probiotics, help inhibit reactive oxygen species production and maintain a healthy intestinal microbiota.[72] Disturbances in gut microbiota composition and reduced fermentation of dietary fibers in IBD might lead to altered SCFA profiles.[71] In a study of IBD patients, reduced tryptophan metabolism was observed, presumed due to altered bacterial communities.[73] Dysbiosis in IBD is marked by an increase in *Enterobacteriaceae*,[74] facultative anaerobes that utilize electron acceptors, such as nitrate, to generate energy. These taxa may impair gut barrier function through inflammatory cytokine production. Consequently, increased *Enterobacteriaceae* alter bile acid metabolism and decrease tight junction integrity, resulting in loss of impermeability in the intestinal epithelium.[36] The beneficial bacterium *Lactobacillus* is decreased in IBD and has shown anti-inflammatory effects in mouse models.[75] In CD, a lower abundance of *F. prausnitzii* signals potential intestinal health issues in adults.[76] *F. prausnitzii* plays an essential physiological role by providing mucosal protection and anti-inflammatory functions.[76] Gut bacterial signatures have been employed as biomarkers, demonstrating associations with mucosal state and related symptoms in UC patients.[77] Certain bacteria, such as *Enterobacteriaceae*, *Klebsiella*, and some *Lachnospiraceae* species, are more abundant in UC patients experiencing symptoms like frequent bowel movements.[77]

Probiotic administration has shown beneficial effects on gut microbiota and therapeutic benefits in various diseases. For example, rectal infusion of *Lactobacillus reuteri* ATCC 55730 improved mucosal inflammation in pediatric patients with distal active UC and altered cytokine expression involved in IBD pathogenesis.[78] Another study found that administration of *Lactobacillus delbrueckii* and *Lactobacillus fermentum* decreased inflammatory cytokines, suggesting probiotics may help prevent UC.[79] The major anaerobic bacterial species in the colon, *Bacteroides*, is reduced in UC; however, administering *Lactobacilli* and *Bifidobacteria* prior to experimental UC induction helped stabilize *Bacteroides* levels, reducing inflammation and tissue damage.[80] A correlation between gut bacterial composition and potential future development of CD was revealed using ML techniques, implying a role for gut bacteria in disease pathogenesis. Based on this, a microbiome risk score was developed, assigning risk scores to individuals based on their gut bacterial profiles, particularly in healthy first-degree relatives of CD patients.[81] These studies demonstrate the potential for using microbial profiles as distinctive markers. By longitudinally tracking changes in these microbial signatures, researchers may predict disease flares or treatment responses.

Extracellular vesicles (EVs) in IBD

EVs are tiny spheres enclosed by lipid bilayers that play a crucial role in the release and transport of various substances, including carbohydrates, lipids, cell wall components, proteins, DNA, RNA, and signaling molecules.[82] Intestinal EVs engage in direct or indirect interactions with immune cells, intestinal epithelial cells, and the gut microbiota, actively participating in the regulation of anti-inflammatory responses, restoration of mucosal barrier integrity, and reconstitution of microbiota composition.[83] EVs are released by various types of cells, such as intestinal epithelial cells, immune cells (including macrophages, T cells, B cells, NK cells, polymorphonuclear neutrophils, and dendritic cells), and the microbiota.[83–85] EVs derived from intestinal epithelial cells maintain gut homeostasis and modulate immune responses.[83] EVs from T cells are involved in intercellular communication and immune modulation.[85] EVs from B cells also contribute to immune responses.[86] By stimulating macrophages, EVs containing inflammasomes can activate the NF-κB pathway, thereby amplifying inflammatory signaling.[87] EVs derived from the microbiota can influence host immune responses and inflammation.[88] Several mechanisms of EV release have been described and warrant further study. For example, plasma membrane budding involves the direct outward budding of the plasma membrane and requires GTPases and the ESCRT complex.[84] EVs can also be released through the fusion of plasma membranes with multivesicular bodies, leading to the release of exosomes.[84] Another mechanism is calcium-dependent release, in which calcium functions as a second messenger and regulator of EV secretion.[89] EVs facilitate intercellular communication by carrying a variety of bioactive molecules, including

Table 1. Organisms of the gut microbiome, their metabolites, and the immune factors they affect

Microbiome list					
Gut bacteria in IBD					
Bacteria	Abundance	Associated metabolite	Metabolite levels	Effect on immune component	References
F. prausnitzii	Decrease	Butyrate	Decrease	Th17/Treg balance disrupted. Blocks IL-6/STAT3/IL-17 pathway and promotes pro- inflammatory effect	37,38
Enterobacteriaceae	Increase	LPS	Increase	Reduced IL-10. Increased IL-8, tumor necrosis factor (TNF)-α, and IL-1β	36
Lactobacilli	Decrease	Indole-3-lactic acid	Decrease	Impairs $CD8^+$ T cells and IL-12a production	39–41
		Exopolysaccharides (EPS)	Decreases	Increased pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6). Decreased anti-inflammatory cytokines (IL-10)	
		Conjugated linoleic acid	Decrease	Production of colonic IL-6 and TNF-α	
Bifidobacteria	Decrease	Indole-3-lactic acid	Decrease	Increased production of IL-8 and TNF-α, and pro-inflammatory cytokines	42,43
Flavobacterium	Decrease	Citric acid	Decrease	Production of TNF-α, IL-6, IL-12 and promotion of pro- inflammation	44,45
		Trimethylamine-N-oxide (TMAO)	Increase	Impacts ATG16L1-induced autophagy. Activates NLRP3 inflammasome. Promotes inflammation	44,46
Bacteroidetes	Decrease	SCFA	Decrease	Reduced Treg cells	47
E. coli	Increase	LPS	Increase	Increased IL-8 and other pro-inflammatory cytokines	35,48,49
Mycobacterial species	Increase	SCFA	Decreases	Reduced suppression of NF-κB; Reduced activation of inflammasomes; Reduced Treg cells. Reduction in anti-inflammatory mediators (TGF-β and IL-10)	35,50,51
Anaerostipes	Decrease	Butyrate	Decreases	Increase in NF-κB-induced pro-inflammatory cytokines TNFα and IL6	52,53
Methanobrevibacter	Decrease	Methane	Decreases	Increases IL-6, TNF-α, IL-1β, IFN-γ, NF-κB	54–56
Christensenellaceae	Decrease	Acetate, Butyrate	Decreases	Increase in IL-8, NF-κB activation	57
Ruminococcus gnavus	Increase	Glucorhamnan polysaccharide	Increases	Increases TNFα	32
Prevotella copri	Decrease	Valerate and other SCFA	Decreases	Increases TNFα	58–61
Clostridium leptum group (IV)	Decrease	Butyrate	Decreases	Reduced Treg differentiation. Reduced IL-22 production. Promotes inflammation through LPS-induced NF-kβ activation. Increased pro-inflammatory factors	62,63
Gut mycobiome in IBD					
C. albicans	Increase	SCFA	Decrease	Increases IL-17 and IL-23 production	64,65
Malassezia	Increase	FFAs such as oleic acid, cell wall carbohydrates, indoles	Increase	Increase production of IL-17 IL-18, IL-8, and IL-6 and Th22 chemokines	64,66
Kluyveromyces	Decrease	β-glucan	Decreases	Reduction in Treg and IL-10	64,67,68

IBD, inflammatory bowel disease; LPS, lipopolysaccharide; SCFA, short-chain fatty acids; EPS, exopolysaccharides; TMAO, trimethylamine-N-oxide; FFAs, free fatty acids; Th17, T-helper 17 cells; Treg, regulatory T cells; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-12, interleukin-12; IL-12a, interleukin-12 subunit alpha; IL-17, interleukin-17; IL-18, interleukin-18; IL-22, interleukin-22; IL-23, interleukin-23; TNF-α, tumor necrosis factor-alpha; IFN-γ, interferon-gamma; TGF-β, transforming growth factor-beta; STAT3, signal transducer and activator of transcription 3; NF-κB, nuclear factor kappa B; ATG16L1, autophagy-related 16 like 1; NLRP3, NLR family pyrin domain containing 3 (inflammasome).

Table 2. Changes in the abundance of the gut virome in IBD

Gut virome	Abundance	Role	References
Myoviridae	Increase	These are temperate viruses belonging to the order *Caudovirales*, but their function in IBD virome remains mostly unclear	101
Microviridae	Decrease	Belonging to the order *Petitivirales*, impacts on bacterial dysbiosis	102
Siphoviridae	Increase	Temperate viruses belonging to the order *Caudovirales*, impacts on bacterial dysbiosis	101,103
Quimbyviridae	Decrease	Role in IBD remains unclear	101
Genomoviridae	Increase	Belonging to order *Geplafuvirales,* the role remains unclear	101
Anelloviridae	Increase	They are useful for reporting reduced immune surveillance and the effectiveness of immunosuppression	104
Retroviridae	Increase	The overgrowth of this family has been linked to several diseases, including CD, but its exact role remains unclear	101

IBD, inflammatory bowel disease; CD, Crohn's disease.

nucleic acids, proteins, lipids, and metabolites, which can initiate intracellular signaling pathways.[90,91] These cargoes can include tricarboxylic acid (TCA) cycle intermediates, steroid hormones, sterols, enzymes, signaling proteins, surface receptors, mRNAs, microRNAs (miRNAs), sphingolipids, and phospholipids. EVs can also elicit responses in recipient cells by initiating intracellular signaling pathways.[90,91]

Recent studies have found that circulating miRNAs contained in EVs are small, non-coding RNA molecules that can serve as biomarkers for diseases.[92] In the context of IBD, miRNAs are of particular interest as potential biomarkers. For example, elevated expression of circulating miRNAs has been detected in EVs isolated from the serum, plasma, or peripheral blood of individuals with IBD.[93] Proteomic analyses comparing EVs from individuals with IBD and healthy controls have identified certain proteins, including ANXA1 and PSMA7, that are enriched in the EVs of IBD patients.[94,95] Notably, levels of EV-associated PSMA7 were found to be lower in IBD patients in remission compared to those with active disease, highlighting the potential utility of EV-based biomarkers for monitoring disease progression in IBD.[95] By addressing various aspects, such as release mechanisms, signaling pathways, and the role of EVs as biomarkers, a comprehensive understanding can be developed of how EVs contribute to the pathophysiology of IBD.

Gut virome

Viruses are increasingly recognized as integral components of the human microbiome, serving diverse ecological functions, including preying on bacteria, stimulating the immune system, facilitating genetic diversity, enabling horizontal gene transfer, fostering microbial interactions, and enhancing metabolic processes.[96] The interaction between bacteria and viruses in the gut highlights the role of viruses in maintaining gut equilibrium and influencing pathological states.[97] The healthy human gut virome includes members of the *Malgrandaviricetes* (spherical ssDNA) and *Caudoviricetes* (tailed dsDNA) phage classes.[98] Although the bacterial hosts of many of these viruses remain largely unidentified, *Caudoviricetes* are presumed to infect a diverse array of bacterial phyla, including *Bacteroidetes, Verrucomicrobia, Proteobacteria, Firmicutes*, and *Actinobacteria*.[99]

Recently, important associations have been observed between the gut virome and IBD. Virome dysbiosis has been implicated in IBD pathogenesis, with elevated levels of phages infecting *Clostridiales*, *Alteromonadales*, and *Clostridium acetobutylicum* detected in individuals with IBD compared to healthy subjects.[100] A study on Chinese cohorts of IBD patients and healthy controls identified 139 IBD-associated viral OTUs, noting a higher abundance of the *Retroviridae* family in IBD patients[101] Additionally, IBD patients showed increased evenness and richness in their eukaryotic virome compared to healthy controls. *Genomoviridae* and *Retroviridae* were two eukaryotic viral families found to be enriched in IBD patients. In contrast, the prokaryotic virome in IBD patients displayed significantly decreased diversity. Families such as *Siphoviridae* and *Myoviridae* were enriched in patients, while crAss-like phages and *Quimbyviridae* were decreased. At the OTU level, numerous IBD-enriched *Siphoviridae* and *Myoviridae* viral OTUs were identified, which infect bacteria such as *Escherichia*, *Klebsiella*, and other opportunistic pathogens known to induce inflammation and trigger disease. Moreover, fecal virome transplantation in mouse models has shown that colonization by these IBD-associated viruses can modulate experimental colitis.[101] However, more comprehensive and focused research is required to achieve a detailed understanding of the virome's role in IBD (Table 2).[101–104]

Gut mycobiome

Fungi make up approximately 0.1% of the gut microbiome and have been identified in the GI tract of around 70% of healthy individuals. They interact with both viruses and bacteria in the gut, exhibiting antagonistic and synergistic relationships.[105] The healthy human GI tract mycobiome is primarily composed of three major phyla: *Basidiomycota, Ascomycota*, and *Chytridiomycota*.[105] Dysbiosis in the fungal community plays a crucial role in IBD by altering gut microbiota composition or promoting the production of pro-inflammatory cytokines.[106] A distinctive feature of IBD is an increased *Basidiomycota*-to-*Ascomycota* ratio (Table 3).[64,66,107–110] An increase in the abundance of fungi such as *Candida* species, which can exacerbate inflammation, and a decrease in Saccharomyces have been observed in IBD cases.[108–110] Another report indicated elevated abundances of *Sterigmatomyces*, *Aspergillus, Candida*, and *Wickerhamomyces*, along with lower abundances of *Penicillium, Exophiala, Alternaria, Acremonium, Trametes, Epicoccum*, and *Emericella* in patients with UC.[111] In a clinical study involving colon biopsies from 10 IBD patients and 18 healthy controls, *Pseudomonas* was elevated, and the opportunistic pathogen *Malasseziales* was found to be the most abundant in UC. An increased *Basidiomycota*-to-*Ascomycota* ratio was also observed in UC compared to CD, due to the higher abundance of *Malasseziales*, which may serve as an indicator of UC.[112] Fungal

Table 3. Changes in the abundance of the gut mycobiome in IBD

Abundance		
Increased	**Decreased**	**References**
C. albicans; Malassezia; Filobasidiaceae; D. hansenii; Xeromyces; Rhodosporium; Lipomyces; Yamadazyma; Yamadazyma friedrichii; Lipomyces doorenjongii	*Saccharomyces cerevisae; Saccharomyces boulardii; Kluyveromyces; C. tropicalis; Zygomycota; Aspergillus; Debaromyces; Cladosporium; Microdochium; Phaeosphaeria; A. rubrobrunneus*	64,66,107–110

dysbiosis has been shown to promote IBD by enhancing $CD4^+$ T cell responses in a mouse model, as well as in human colonic and $CD4^+$ T cell samples from healthy donors, UC patients, and CD patients. In this report, *Candida albicans* was found to increase pro-inflammatory cytokine production, and slower progression of IBD was observed when terbinafine was used to deplete fungi.[113]

Correlating microbial signatures with disease outcome and treatment response

When discussing microbial signatures in IBD, it is important to highlight the specific compositional changes in the gut microbiota associated with disease activity, progression, and treatment response. In patients with IBD, the intestinal microbiota is dysregulated compared to that of healthy individuals, showing decreased bacterial diversity—particularly reduced abundances of *Firmicutes* and *Bacteroidetes*—and an increase in *Proteobacteria*.[114] Differences also exist between CD and UC. Patients with CD exhibit a higher level of dysregulation, characterized by reduced microbial diversity and stability, which can be considered a specific signature of CD.[115] Microbial biomarkers, such as specific bacterial strains or metabolites, can indicate whether a patient is likely to respond to a particular treatment. For instance, in patients with CD treated with the anti-integrin therapy vedolizumab, those who achieved remission had a gut microbiome enriched with *Roseburia inulinivorans* and a *Burkholderiales* species compared to non-responders.[116] Similarly, in pediatric IBD patients, a higher absolute abundance of *Bifidobacteriales* and a lower abundance of *Actinomycetales* at baseline were associated with a rapid response to infliximab therapy.[117] Additional studies like these will help us better understand the microbial signatures associated with IBD and support the development of personalized treatment strategies, ultimately improving patient outcomes.

Metabolome signatures in IBD

The metabolome refers to the pool of small metabolites present in a biological sample under specific conditions at a particular time.[118] Important metabolites include lipids, amino acids, and TCA cycle intermediates, among others. A diverse range of biosamples, including easily accessible fluids such as blood, urine, serum, feces, and saliva, as well as less accessible and more invasive samples such as organs, tissues, or cells, has been used to identify metabolomic markers in IBD.[119] These samples provide varying degrees of information: for example, urine provides a comprehensive overview of endogenous and exogenous metabolism; stool gives insight into digestive metabolism; blood offers a systemic perspective; tissue samples provide direct information on localized pathology; and less conventional samples like breath may reflect dynamic metabolic processes.[120]

Several studies have reported variations in metabolites from different types of biosamples in patients with IBD compared to healthy controls. For example, in a large cohort of 117 individuals with CD, increased levels of 1-octen-3-ol, 6-methyl-2-heptanone, 2-piperidinone, and heptanal were observed in fecal samples from the active CD group compared to healthy controls. Conversely, reduced quantities of methanethiol, 3-methyl-phenol, SCFAs, and ester derivatives were observed in CD patients.[121] Another study found that fecal metabolites could distinguish between UC and CD, as well as between healthy controls and UC. Fecal samples from CD patients showed enrichment in lactate, succinate, alanine, and tyrosine, while UC patients exhibited higher levels of leucine, alanine, and tyrosine.[122] In yet another study, 53.6% of lipid metabolites were significantly altered in CD compared to controls, whereas only five lipid-related metabolites were decreased in UC. Both CD and UC exhibited consistent decreases in various fatty acids compared to controls. Interestingly, glycerol levels were notably reduced in CD, indicating lipolysis. Essential acylcarnitine metabolites were also reduced in CD compared to both UC and control groups. Bile acid pathways were significantly altered in IBD, with increased primary and secondary bile acids observed in CD, while UC displayed reduced primary bile acids and altered secondary bile acids. TCA cycle intermediates, including citrate, aconitate, α-ketoglutarate, succinate, fumarate, and malate, were significantly decreased in CD. Additionally, β-hydroxybutyrate, derived from acetyl-CoA, showed the most substantial reduction in CD, being 11 times lower than in controls and 18 times lower than in UC subjects.[123]

Metabolic dysfunction is defined as a series of abnormal or disrupted metabolic processes occurring in the body, particularly those related to energy production, nutrient utilization, or the regulation of various molecules. In IBD, this dysfunction is marked by decreased levels of trimethylamine-N-oxide, reduced SCFAs like butyrate, lower hippurate levels, and alterations in primary and secondary bile acid profiles.[120] This dysbiosis can contribute to inflammation by influencing metabolic pathways or the immune system.[124] Several metabolites in the gut are derived from the resident microbiota. Therefore, microbial dysbiosis is expected to be associated with metabolic imbalance. For example, in IBD, there is an imbalance in microbial composition, with an increase in *Proteobacteria* and a decrease in *Firmicutes*. Thus, the roles of specific bacteria, such as *E. coli* and the butyrate-producing *F. prausnitzii*, become particularly important. This suggests that combining microbiome and metabolome analyses may provide valuable insights for understanding, diagnosing, and treating IBD.[120] Supporting this idea, Lijun Ning *et al*. (2023) identified unique biomarkers related to IBD that consist of specific gut bacteria and metabolites. These biomarkers are expected to have a low likelihood of being incorrectly identified in both GI and non-GI diseases, suggesting their potential as valuable disease-specific markers for IBD with diagnostic utility.[125]

Metabolomic profiling has also proven valuable in predicting treatment responses in IBD patients by identifying specific metabolites associated with therapeutic outcomes. Key metabolites such as bile acids, glycine, linoleic acid, N-acetylserotonin, and

methylglutaric acid have been linked to responses to therapies like anti-TNF and infliximab, with distinct profiles observed between responders and non-responders. Notably, bile acids, along with urinary cysteine and bile acids measured in various bodily fluids, have emerged as potential indicators of treatment efficacy. Furthermore, fecal lipid profiles have shown higher predictive accuracy than serum profiles. These findings emphasize the potential of metabolomic analyses, particularly of fecal samples, to enhance personalized treatment strategies and improve understanding of IBD's metabolic alterations.[126]

To explore metabolite profiles, it is essential to implement highly sensitive techniques for metabolomics analysis. The two key analytical techniques used in metabolomics are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). NMR provides insights into metabolite structure and concentration by detecting energy changes in nuclei under a magnetic field. It includes 1D-NMR, which is commonly used in high-throughput studies, and 2D-NMR, which resolves overlapping peaks for complex metabolite characterization using techniques such as COSY and TOCSY.[127] While NMR faces limitations such as low sensitivity and overlapping proton peaks, it is expected to remain a vital tool in healthcare for at least the next decade due to advantages including minimal sample preparation, high reproducibility, and the ability to analyze an entire sample in a single measurement.[128]

MS, on the other hand, measures the mass-to-charge ratio (m/z) of ionized metabolites, offering high sensitivity and specificity. MS is often coupled with chromatographic separation (e.g., liquid chromatography-MS or gas chromatography-MS) to reduce sample complexity and improve accuracy.[127] However, structural elucidation in untargeted analyses presents significant challenges, particularly with stability and reproducibility during large-scale sample runs using untargeted liquid chromatography-MS. These issues underscore the need for advancements that enhance reproducibility and improve the accuracy of structure elucidation.[128]

In IBD research, untargeted metabolomics is particularly valuable as it captures a holistic metabolic profile without predefined targets. The workflow includes spectral processing to generate metabolic features, data analysis to explore associations with phenotypic traits, biomarker discovery for diagnostics, and pathway analysis to connect metabolites with biological processes. Advanced bioinformatics tools are essential for managing the complexity of metabolomics data, enabling multi-omics integration, and identifying biomarkers and pathways relevant to IBD. Together, these techniques provide a comprehensive understanding of disease mechanisms and support the development of personalized therapeutic strategies.[129]

Immunological biomarkers in IBD

The need for precise diagnostic tools and effective treatment strategies for IBD highlights the importance of immunological biomarkers. Intensive research aimed at identifying these biomarkers, coupled with the vast amount of related data being generated, now enables speculation about the dynamics between immunological components and the gut microbiome. Understanding how dysbiosis in the gut microbiome can affect the regulation of various immune system components is crucial for elucidating the mechanisms by which the disease progresses. Numerous studies have identified different immunological biomarkers, including various types of T cells, receptors, antibodies, ILs, and cytokines. These biomarkers can be classified according to their clinical applications: diagnosis, prognosis prediction, and treatment monitoring.

Diagnosis

Immunological biomarkers are essential for the accurate diagnosis of IBD, helping to differentiate it from other GI disorders, particularly in cases where clinical symptoms are ambiguous. Specific biomarkers, such as anti-Saccharomyces cerevisiae antibodies and perinuclear anti-neutrophil cytoplasmic antibodies, are commonly used to distinguish between CD and UC, respectively.[130] These markers provide less invasive diagnostic options and complement traditional methods like endoscopy. Elevated levels of pro-inflammatory cytokines, including IL-1β and IL-8, have been identified in IBD patients, aiding in the identification of active inflammation. IL-1β is a biomarker noted for its elevated levels in the serum of patients with IBD, in those experiencing relapse, and in pediatric cases.[131,132] IL-1β alone is not capable of exacerbating IBD, but its effect is mediated through fluctuations in the levels of inflammasomes like NLRP3, an important source of mucosal IL-1β.[133] Lower levels of the NLRP3 inflammasome, and consequently lower levels of IL-1β, lead to a decreased state of colitis in mice.[134] Furthermore, in mice, aggravated intestinal inflammation is linked to bacterial members of the genus *Prevotella*, mainly *Prevotella intestinalis*, which can reduce the production of SCFAs, especially acetate.[135] Interestingly, acetate is now known to suppress NLRP3 inflammasome-mediated production of IL-1β.[136] From these studies, it can be understood that in severe cases of intestinal inflammation, *Prevotella* species reduce acetate production, which in turn increases IL-1β production and promotes inflammation. Similarly, IL-8 levels are elevated in the serum of patients with IBD relapse and in pediatric cases.[131,132] The increase in IL-8 levels in CD and UC can be explained by the higher abundance of various *Enterobacteriaceae*, such as *E. coli*, and the reduced abundance of *Bifidobacteria* and *Lactobacillus* species in the gut.[137–139] A study using IBD isolates showed that flagellin shed by mucosa-associated *E. coli* induces IL-8 expression through a MAPK-dependent pathway.[139] *Bifidobacterium* species are also known to bind and neutralize lipopolysaccharides (LPS) from *E. coli*, which, if not neutralized, can lead to LPS-induced increases in IL-8 levels.[140] It is possible that due to the increased levels of *E. coli* and lower levels of *Bifidobacteria*, LPS produced by *E. coli* is not effectively neutralized, leading to more LPS-induced IL-8 production. *Bifidobacterium* is also an SCFA-producing bacterium, and these SCFAs exert an anti-inflammatory effect by blocking the NF-κB signaling pathway. Thus, low levels of Bifidobacteria in IBD, and consequently lower production of SCFAs, can result in increased pro-inflammatory consequences.[33]

Similarly, an experiment in IBD patients demonstrated that an increase in *Lactobacillus* species, and thus increased butyric acid production, leads to decreased levels of IL-8 and other pro-inflammatory molecules.[141] From this, we can infer that lower levels of *Lactobacillus* may contribute to elevated IL-8 levels. Dysbiosis in the gut microbiome, characterized by altered levels of bacterial species such as *Prevotella intestinalis* and *E. coli*, further influences the production of these cytokines, reinforcing their diagnostic relevance.

Prognosis prediction

Immunological biomarkers are instrumental in predicting the progression of IBD and identifying patients at risk for severe disease or complications. For instance, Oncostatin M (OSM), which belongs to the IL-6 family of cytokines, has been shown to be an important biomarker in IBD.[142] It is rapidly released during degranulation and can initiate other signaling pathways, such as the JAK-STAT and PI3K-Akt pathways, which promote disease pro-

gression.[142] Mucosal OSM levels have been found to be upregulated in patients newly diagnosed with IBD and in those experiencing relapse, even after surgery.[142] High levels of OSM have been identified in patients with both CD and UC, and have also been shown to predict non-responsiveness to anti-TNFα therapy.[143] Dysbiosis in CD has been associated with a decreased abundance of *Roseburia intestinalis*, a gut microbiome member, and reduced levels of this species are linked to increased OSM levels in CD patients.[144] Interestingly, normal *R. intestinalis* abundance can suppress intestinal inflammation by downregulating pro-inflammatory cytokines and increasing anti-inflammatory cytokines and regulatory T cells (Tregs).[144] This effect can be explained by the fact that *R. intestinalis* is an SCFA-producing bacterium capable of synthesizing butyrate, which has multiple effects on immune regulation, including promoting the proliferation of Tregs.[145] From this, it can be understood that in IBD, dysbiosis results in decreased *R. intestinalis* abundance, leading to reduced butyrate production, downregulation of Tregs and anti-inflammatory factors, and upregulation of pro-inflammatory mediators, including OSM.

Treatment monitoring

Biomarkers also play a critical role in monitoring treatment response and guiding therapeutic decisions in IBD. A recent study was performed to identify IBD remission- or relapse-specific biomarkers. A list of ILs, cytokines, and other immunological factors, including Galectin-1, IL-15, IL-21, IL-25, IL-13, IFN-β, CXCL11, CXCL9, CXCL10, and G-CSF, whose levels are elevated in patients with relapse.[132] The possibility that higher levels of IL-15 could result from changes in the levels and composition of the Bacteroidetes and Firmicutes phyla, as well as decreased abundance of butyrate-producing bacteria (leading to reduced butyrate and other SCFA levels), has already been highlighted.[146] The link between lower levels of butyrate and other SCFAs with elevated IL-15 levels may be similar to the mechanisms observed for IL-8 and IL-1β. An experiment performed on patients with hepatocellular carcinoma showed that increased levels of gram-negative bacteria and decreased levels of gram-positive bacteria, such as *Firmicutes*, are associated with elevated IL-25 levels.[147] The increased levels of IL-25 observed in IBD may similarly result from dysbiosis. In IBD, significantly higher levels of the bacteriophage *Caudovirales* have been observed; combined with bacterial infection (where these phages replicate within bacteria), this can trigger pro-inflammatory effects, heightened T cell immune responses, and IFN-β production, while suppressing phagocytosis and TNF production, thereby maintaining gut inflammation.[148] It is now known that CXCL9, CXCL10, and CXCL11 have strong bactericidal activity, and their increased levels may be linked to the higher abundance of *E. coli* in IBD, potentially resulting in infections by bacteria such as *Listeria monocytogenes* and *Bacillus anthracis*.[149–151]

Another study of CD and UC patient samples using mass cytometry identified immunological molecules characteristic of IBD samples, including $CXCR3^+$ plasmablasts, $HLA\text{-}DR^+CD38^+$ T cells, and $IL1B^+$ macrophages and monocytes. Specifically in UC samples, elevated $IL17A^+$ $CD161^+$ effector memory T cells, $HLA\text{-}DR^+CD56^+$ granulocytes, and reduced type 3 innate lymphoid cells were observed. In CD samples, $IL1B^+$ dendritic cells, $IL1B^+TNF^+IFNG^+$ naïve B cells, $IL1B^+HLA\text{-}DR^+CD38^+$ T cells, and $IL1B^+$ plasmacytoid dendritic cells were identified.[152] How gut dysbiosis influences the levels of these immune components is not yet fully understood and warrants further exploration. Much remains unknown about how changes in the abundance and composition of the gut microbiome lead to higher levels of these biomarkers.

Integration of the gut-microbiome, metabolome, and immunological aspects in IBD

The sections thus far have discussed three types of biomarkers—the gut microbiome, the gut metabolome, and immunological biomarkers—independently. However, it is important to consider the interplay between gut dysbiosis, alterations in metabolite levels, and the consequent increase in pro-inflammatory factors and reduction of anti-inflammatory factors. As already established, IBD is characterized by gut dysbiosis, which constitutes the first group of biomarkers. This imbalance in the abundance of different gut microbiota leads to a corresponding imbalance in the levels of various associated metabolites. Broadly, SCFAs and LPS represent two common groups of metabolites whose levels decrease and increase, respectively, due to gut dysbiosis, forming the second group of biomarkers. Various cytokine levels are affected by these changes in metabolite concentrations. The cytokines whose levels change significantly and form the third group of biomarkers include IL-12, IL-10, IL-8, IL-6, IL-1β, and TNF-α. An overall representation of this integrated concept of the three biomarkers is shown in Figure 2. This concept can be further illustrated by examining *F. prausnitzii*, whose abundance in the gut decreases in IBD. This bacterium is a major butyrate producer, and its reduced abundance leads to a corresponding decrease in butyrate levels.[153] Butyrate, an SCFA, impacts the host immune system, supporting the observation that reduced butyrate levels disrupt the Th17/Treg balance. It also inhibits the IL-6/STAT3/IL-17 pathway and promotes pro-inflammatory effects, ultimately exacerbating the disease.[144] This is just one example of a mechanism linking all three types of biomarkers seen in IBD. Other such mechanisms can be similarly understood from Table 1 and are visually summarized in Figure 3. Considering the interactions mentioned above and those outlined in Table 1, it may be hypothesized that: i) alterations in gut microbiome composition directly or indirectly influence the production of immunological biomarkers in IBD patients; ii) the metabolomic signatures associated with dysbiosis in IBD correlate with specific immunological responses and disease severity; and iii) modulating the gut microbiome may lead to measurable changes in both immunological biomarkers and metabolomic profiles, resulting in improved clinical outcomes for IBD patients. The interplay between the gut microbiome, metabolome, and immune factors in IBD presents numerous complexities and unresolved questions. One significant uncertainty lies in the causal relationships between specific microorganisms, metabolites, and immune responses. While associations have been established, the directionality of these relationships remains unclear. For instance, it is uncertain whether dysbiosis leads to altered immune responses or if inflammation modifies microbiome composition. Alterations in metabolite profiles, as discussed above, have been observed in IBD patients, yet the specific metabolic pathways affected and their implications for immune function are not fully elucidated. The role of metabolites produced by gut bacteria in modulating immune responses involves complex interactions that warrant further investigation. Individual variability in immune responses to microbial and metabolic signals further complicates our understanding of pathogenesis. Factors such as genetics, environmental influences, and prior exposures can significantly affect how the immune system interacts with the microbiome. To address these uncertainties, several research methods and technical approaches could be employed. Conducting longitudinal studies that track changes in microbiome composition, metabolomic profiles, and immunological biomarkers over time could help establish causal relationships. This approach would allow researchers to observe

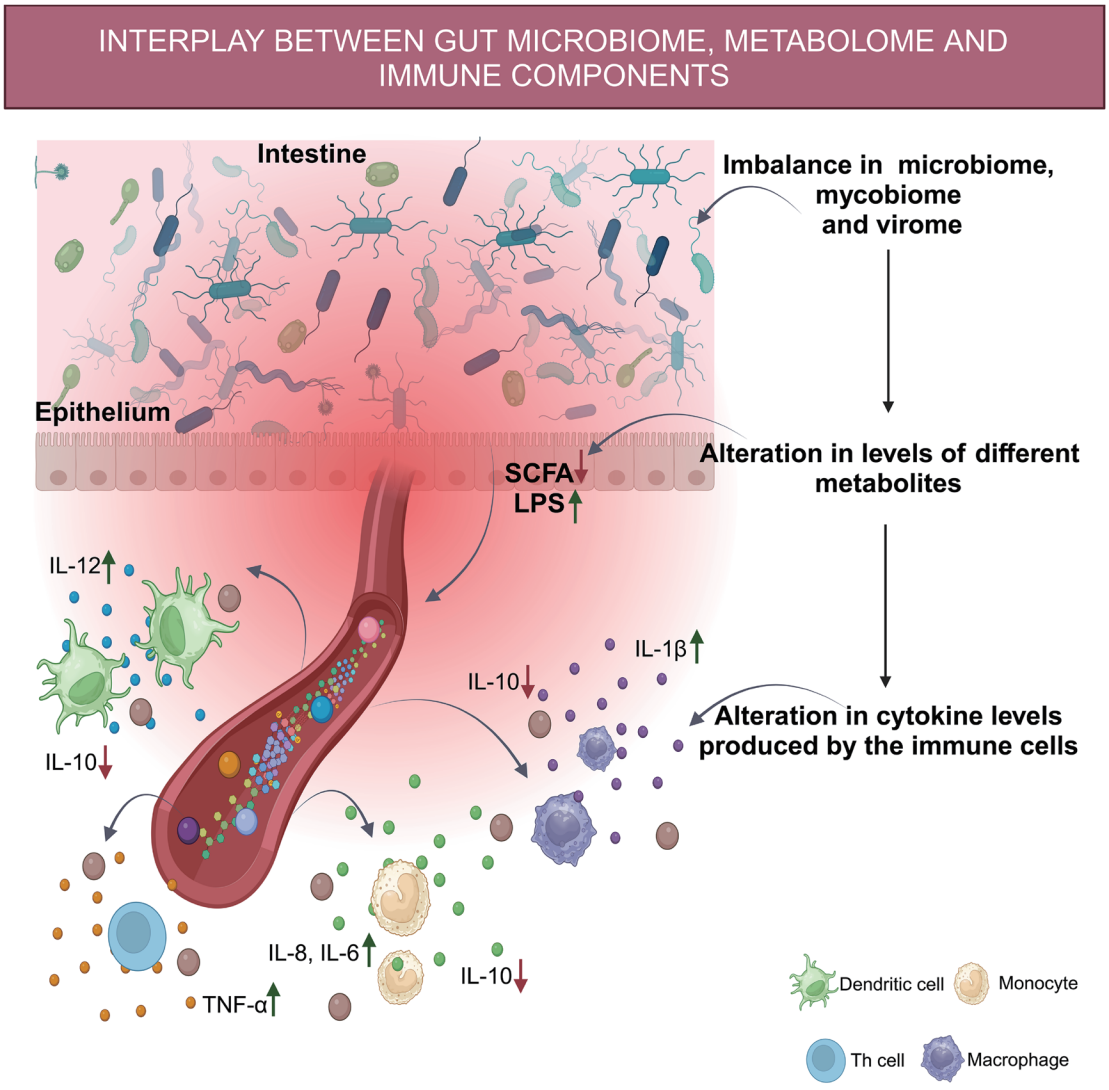

Fig. 2. Dynamics between gut microbiome, metabolome, and immunological components in IBD. Created with Biorender.com. IBD, inflammatory bowel disease; SCFA, short-chain fatty acids; LPS, lipopolysaccharide; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-12, interleukin-12; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1 beta.

how shifts in one domain influence the others. Utilizing an integrative multiomics approach (combining genomics, transcriptomics, proteomics, and metabolomics) can provide comprehensive insights into the interactions between microorganisms, metabolites, and immune factors. Advanced computational models could analyze this data to identify key pathways involved in IBD pathogenesis. Developing animal models that mimic human IBD can facilitate controlled experiments to test specific hypotheses about microbial influence on immune responses. For example, germ-free mice could be colonized with specific bacterial strains to observe subsequent changes in immune activation and metabolite production. Designing clinical trials that monitor immunological biomarkers alongside microbiome and metabolome changes during treatment could provide insights into how therapies influence these interactions. This could lead to personalized treatment strategies based on individual biomarker profiles. These uncertainties need to be addressed through targeted research methods to deepen our understanding of IBD pathogenesis and potentially uncover new therapeutic targets for managing this complex disease.

AI-ML-based advancements

As our understanding of IBD progresses and we strive to enhance clinical trial outcomes and treatment goals, AI and ML have emerged as promising tools to improve diagnostic processes and treatment outcomes. Various ML algorithms, such as Random Forest and Support Vector Machines, have demonstrated efficacy in predicting patient responses to therapies and assessing disease severity.[8] These algorithms utilize high-dimensional data ranging from clinical genomics to microbiome data, enabling a better understanding of individual patient profiles. A study proposes the Holistic AI in Medicine framework, which uses multimodal inputs such as tabular data, time series, text, and images to enhance predictive modeling in healthcare. The integration of

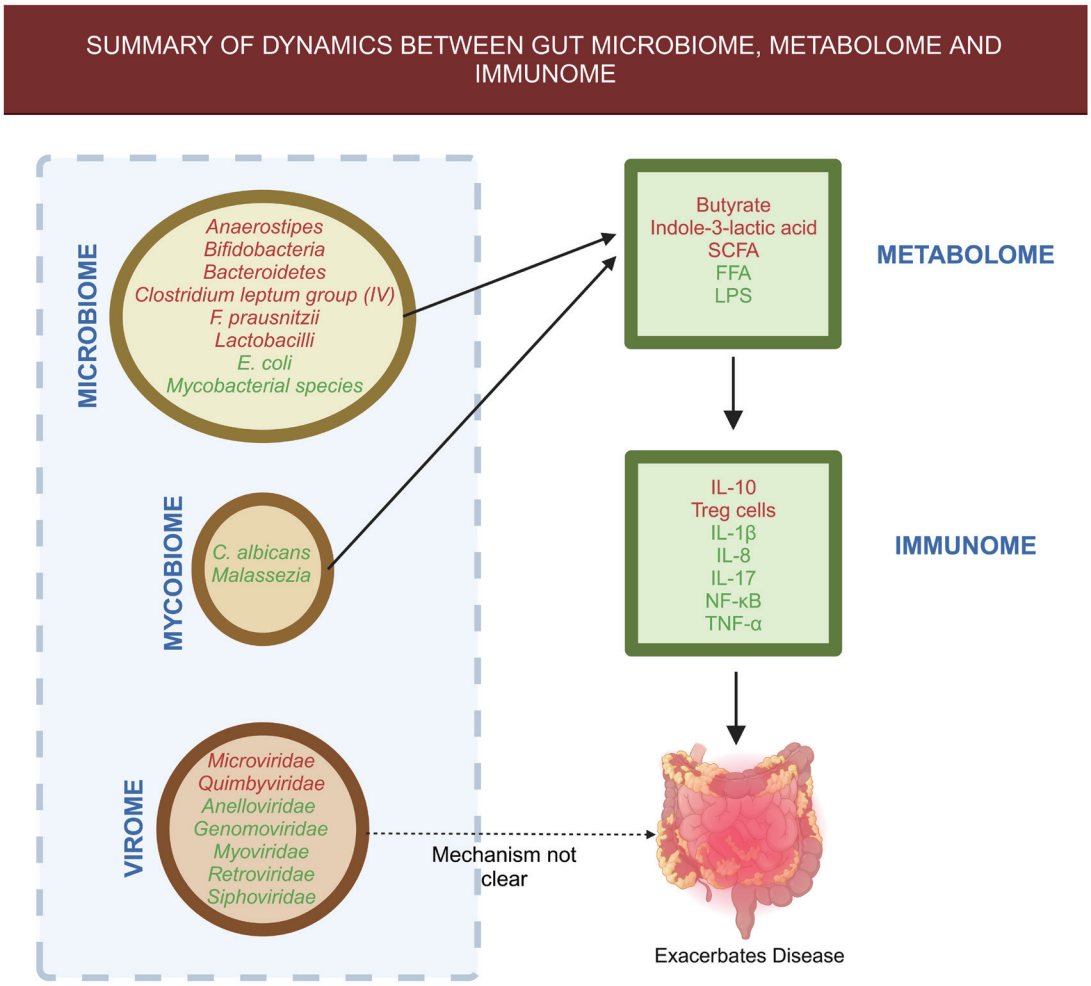

Fig. 3. Visual representation of the interactions between gut microbiome, metabolome, and immunological markers summarized from Table 1 and Table 2. Created with Biorender.com. Text in red and green indicates a decrease and increase in levels, respectively. SCFA, short-chain fatty acids; FFA, free fatty acids; LPS, lipopolysaccharide; IL-10, interleukin-10; Treg cells, regulatory T cells; IL-1β, Interleukin-1 beta; IL-8, interleukin-8; IL-17, interleukin-17; NF-κB, nuclear factor kappa B; TNF-α, tumor necrosis factor-alpha.

multimodal data using AI techniques has been shown to improve diagnostic accuracy.[154] However, many challenges remain unresolved in this field. One major issue is the lack of diversity in patient samples, which can lead to biased predictions. This highlights the need to develop robust AI models that generalize well across populations. Research has shown that AI models trained predominantly on data from specific demographics can result in imbalances in healthcare outcomes, with less accurate algorithms for underrepresented racial or ethnic groups.[155] Moreover, the increasing applications of AI in managing IBD present exciting prospects. AI may not only predict how individuals respond to biological therapies but also contribute to refining the standard of care. This sets the groundwork for personalized treatment in the future, with the potential to reduce costs and improve overall disease management.[156] The integration of AI in the treatment and diagnostics of IBD offers significant potential for enhancing patient care through improved and personalized strategies. To recognize and address these challenges, future research must focus on validating AI systems in real clinical environments using diverse data to optimize the models.

Future research directions

Future research on understanding IBD should focus on conducting large-scale longitudinal studies to explore the intricate relationships between immunological biomarkers, the microbiome, and the metabolome.[157] These studies should aim to recruit a diverse cohort of participants, including those diagnosed with CD and UC, alongside healthy controls for baseline comparisons. Inclusion criteria must consider age diversity and disease duration to effectively evaluate temporal changes in biomarkers, with a target sample size of 500–1,000 participants to ensure robust data analysis.

The study design should adopt a prospective cohort framework, facilitating data collection at multiple time points, such as baseline, six months, and annually thereafter. Regular follow-ups will be essential for gathering biological samples (blood and feces) and clinical data, including symptom diaries and medication use. An intervention group receiving dietary modifications or probiotics could provide valuable insights into their impact on biomarkers over time.

Data collection methods must encompass comprehensive biological sampling for immunological analysis and microbiome sequencing, alongside standardized clinical assessments to evaluate disease

activity. Advanced statistical analyses and ML approaches will be crucial for identifying patterns that predict disease flares or treatment responses based on microbiome and metabolomic changes.[158] Ethical considerations are paramount; thus, obtaining informed consent and ethics approval from relevant boards is essential. Finally, dissemination of findings through publication in peer-reviewed journals and engagement with healthcare providers will enhance awareness of potential biomarkers and their clinical relevance.[159] By implementing these structured strategies, researchers can gain valuable insights into the complex dynamics of IBD, ultimately informing future therapeutic strategies and improving patient outcomes.

Conclusions

In this review, we have summarized biomarkers of IBD with a major focus on signatures in the gut microbiome. We discussed the characteristic variation in the levels of different organisms in the gut, how this variation causes fluctuations in the levels of metabolites produced by these organisms, and finally, how the imbalance of these metabolites can induce altered levels of various immune system components. Thus, this review provides a comprehensive outlook on microbial, metabolic, and immunological signatures and their interrelations. Although extensive research is being conducted to identify biomarkers for IBD, the ones identified so far are not ideal. Most reported biomarkers are invasive, lack specificity to IBD, and are not highly sensitive. Moreover, biomarkers reported across different studies are inconsistent due to variations in study protocols, sample sizes, populations, environmental influences, experimental bias, and other factors. This implies that there is no standardized panel of biomarkers that can be universally applied across all populations and stages of IBD. The biomarkers known so far need to be validated through longitudinal studies across diverse, larger populations using various study designs and heterogeneous patient samples. This will help establish standard biomarkers usable in all IBD cases. These biomarkers can then be used for timely prognosis, accurate diagnosis, and can also inform personalized treatment strategies for patients.

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Conflict of interest

The authors have no conflict of interest related to this publication.

Author contributions

Study concept and design (PSP, RK, VS, TP), creating tables and figures (RK, PSP, VS), drafting of the manuscript (PSP, RK, VS), critical revision of the manuscript for important intellectual content (TP), and study supervision (TP). All authors have made significant contributions to this study and have approved the final manuscript.

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