Received: 14 July 2022 | Accepted: 5 November 2022

DOI: 10.1002/ueg2.12338

REVIEW ARTICLE

UNITED EUROPEAN GASTROENTEROLOGY ueg journal WILEY

The role of gut microbiome in inflammatory bowel disease diagnosis and prognosis

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Abstract

Inflammatory bowel disease (IBD) is a chronic immune-mediated intestinal disease consisting of ulcerative colitis and Crohn's disease. Inflammatory bowel disease is believed to be developed as a result of interactions between environmental, immune-mediated and microbial factors in a genetically susceptible host. Recent advances in high-throughput sequencing technologies have aided the identification of consistent alterations of the gut microbiome in patients with IBD. Preclinical and murine models have also shed light on the role of beneficial and pathogenic bacteria in IBD. These findings have stimulated interest in development of non-invasive microbial and metabolite biomarkers for predicting disease risk, disease progression, recurrence after surgery and responses to therapeutics. This review briefly summarizes the current evidence on the role of gut microbiome in IBD pathogenesis and mainly discusses the latest literature on the utilization of potential microbial biomarkers in disease diagnosis and prognosis.

KEYWORDS

biomarker, Crohn's disease, inflammatory bowel disease, microbiome, ulcerative colitis

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory disease which typically includes two subtypes, Crohn's disease (CD) and ulcerative colitis (UC). Over the past few decades, the incidence of IBD in the West including Europe and North America has stabilized whereas the incidence in newly industrialized countries has continued to climb at a rapid rate.[1–3] Although the etiology of IBD is not completely understood, it has been reported that disease pathogenesis is related to changes in host genetics,[4,5] mucosa immunity,[6,7] environmental factors[8] and the gut microbiome.[9,10] Epidemiological studies have shown that environmental exposures such as diet, cigarette smoking, hygiene status, antibiotic use, mode of birth and breastfeeding[11] may contribute to disease pathogenesis of IBD in part via alterations of the gut microbiota.[12–17] Recent advances in high-throughput sequencing with more rapid and less costly microbial sequencing of biosamples have allowed more comprehensive delineation of microbial gene and pathway composition as well as integrative network analyses between different microbial communities.[18]

Several studies have shown that patients with IBD have perturbed and dysregulated intestinal microbiota compared with healthy subjects.[19–21] Microbial biomarkers are emerging as promising non-invasive tools to predict disease risk, disease activity, disease course, recurrence after surgery and responses to therapeutics.[11] This review briefly summarizes the current evidence on the role of

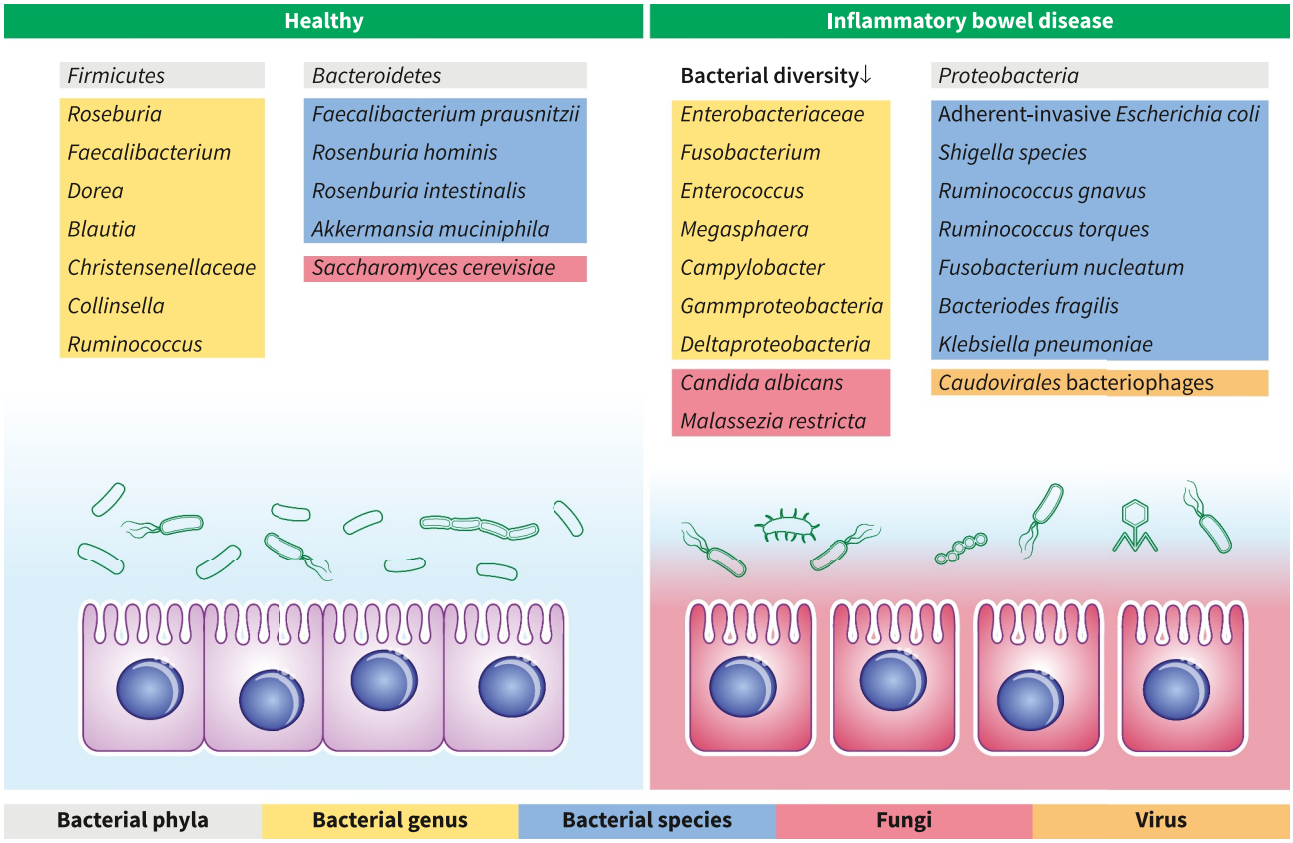

FIGURE 1 Microbial alterations in IBD. The gut microbiome in IBD patients is generally characterized by a decrease in bacterial diversity, decrease in abundance of *Firmicutes* and *Bacteroidetes*, and an increase in *Proteobacteria*. Alteared bacteria at genus and species level, fungi, virus are shown in the figure. IBD, inflammatory bowel disease

gut microbiome in IBD pathogenesis (Figure 1) and mainly discusses the latest literature on the utilization of potential microbial biomarkers in disease diagnosis and prognosis (Figure 2).

THE ROLE OF GUT MICROBIOME IN IBD

Preclinical and clinical studies have shown an important role of gut dysbiosis in IBD pathogenesis. In animal studies, a germ-free environment prevents development of inflammation in genetically susceptible mice,[22] whereas the transfer of proinflammatory microbiota from diseased mice into healthy mice induced inflammation.[23] Furthermore, colonization of mice with fecal microbiota from patient with IBD worsened colitis by altering the gut microbiota.[24] In human subjects, disease activity is most obvious in areas where bacterial populations are highest (the colon) and where there is stasis of feces (terminal ileum and rectum). In addition, fecal diversion improves inflammation but restoration of bowel continuity leads to disease recurrence.[25,26] Antibiotics have proven to be effective in some patients with CD and specific bacteria have been reported to drive or suppress intestinal inflammation.[27]

To date, fecal samples are the most commonly used sample type to depict the gut microbiome as optimized methods to process mucosal microbiota has been more challenging. The gut microbiome of IBD patients is generally characterized by reduced diversity, decrease in abundance of *Firmicutes* and *Bacteroidetes*, and an increase in *Proteobacteria*. At the genus level, patients with IBD commonly lack beneficial bacteria, such as *Roseburia*, *Faecalibacterium*, *Dorea*, *Blautia*, *Christensenellaceae*, *Collinsella*, *Ruminococcus* and other butyrate-producing bacteria.[28] Conversely, bacterial groups such as *Enterobacteriaceae*, *Fusobacterium*, *Enterococcus*, *Megasphaera*, *Campylobacter* and sulfate-reducing *Gammaproteobacteria* and *Deltaproteobacteria* were shown to be expanded in stool and mucosa of patients with IBD.[9,29]

Within the class *Clostridia*, several studies have reported a decrease in the *Clostridium leptum* groups, particularly *Faecalibacterium prausnitzii*. It has been reported that *F. prausnitzii* with anti-inflammatory properties is depleted in both mucosal and fecal samples in patients with UC and CD.[30,31] *Roseburia*, a clade of *Clostridia* XIVa group, is an acetate utilizer and butyrate producer. *Roseburia hominis* was found to be negatively correlated with disease activity in UC patients. *Roseburia intestinalis* has been shown to produce butyrate and induce anti-inflammatory responses to alleviate experimental colitis.[32] Within *Verrucomicrobiota* phylum, *Akkermansia muciniphila*, a mucus-degrader that was found to colonize the gut could induce the production of homeostatic IgG and prevent pathogenic bacteria from multiplying.[33]

Adherent-invasive *Escherichia coli* and *Shigella* have been consistently reported to be increased in fecal and mucosal samples of IBD

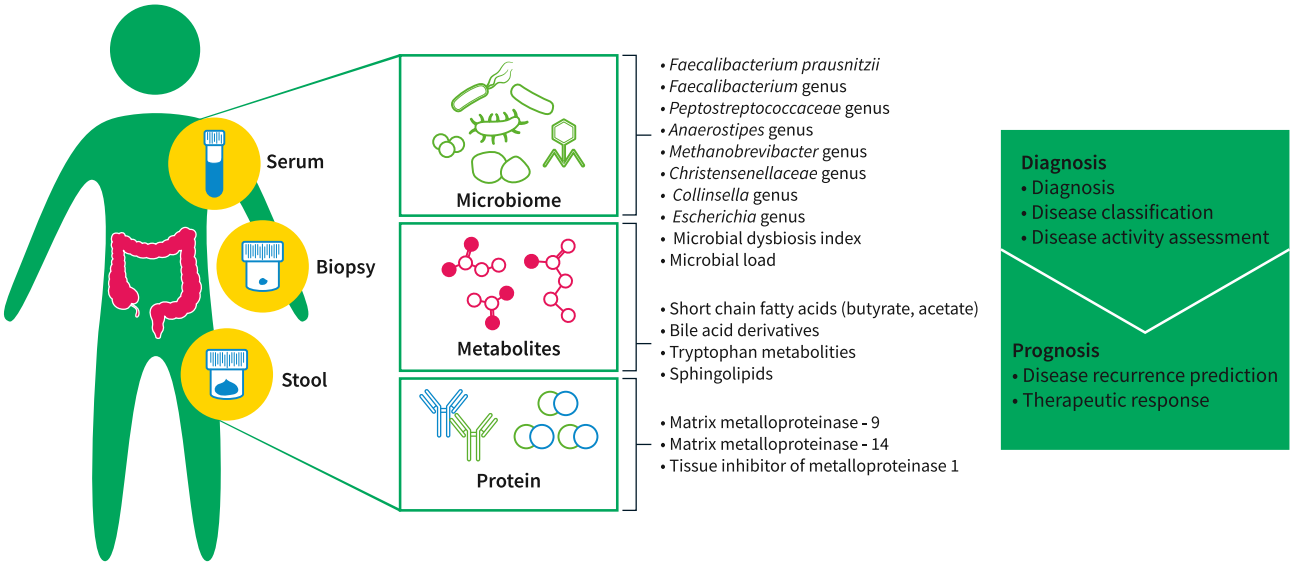

FIGURE 2 Microbiome-associated biomarkers in IBD diagnosis and prognosis. Fecal and mucosal microbiome, including bacteria, fungi, virus, are useful in IBD diagnosis, classification, disease activity, disease course, recurrence after surgery and responses to therapeutics. Bacteria derived metabolites, and serum and fecal microbe-associated proteins are applied in IBD determination and classification. IBD, inflammatory bowel disease

patients.[34] *Ruminococcus gnavus* and *Ruminococcus torques*, commonly known as colon-associated mucolytic bacteria, were abundant in the gut of IBD patients.[35,36] *Ruminococcus gnavus* produced inflammatory polysaccharide and induced the secretion of inflammatory cytokines such as TNF-α by dendritic cells.[37] *Fusobacterium nucleatum* was frequently found in the gut of IBD patients and their presence were associated with reduced overall fecal microbial diversity, and their abundance also correlated with disease activity. Studies have indicated that *F. nucleatum* can damage intestinal epithelium and trigger inflammation in the gut.[38,39] *Bacteroides fragilis*, especially the enterotoxigenic *B. fragilis*, were found to be of higher prevalence and abundance in fecal samples of patients with IBD than that in healthy controls.[40,41] Recently, a potential pathobiont, *Proteus mirabilis* is positively correlated with Crohn's disease activity score (CDAI) and associated with Crohn's disease pathogenesis by activating pro-inflammatory pathways in germ-free mice.[42] The prevalence and abundance of *P. mirabilis* were also higher in fecal and mucosal samples of patients with CD than that in controls. A recent study[43,44] found that *Klebsiella pneumoniae* (Kp) was enriched in the gut microbiota of IBD patients across geography. Isolated Kp strains induced severe intestinal inflammation and tissue damage, suggesting that the Kp strains may contribute to worsening IBD.

The composition and diversity of fungi and viruses in the gut microbiome are also altered in patients with IBD.[45,46] *Candida albicans* was reported to be enriched while *Saccharomyces cerevisiae* was depleted in the faeces of IBD patients and the mucosa of CD patients.[47–49] *Malassezia restricta*,[50] the common skin resident fungus, is significantly increased in the mucosal samples of patients with CD. Fecal samples analysis showed that the gut virome of patients with IBD was associated with an expansion of *Caudovirales* bacteriophages[51] and rectal samples in patients with UC had an increased abundance of *Caudovirales* bacteriophages with a reduction in bacterial diversity which correlated with intestinal inflammation.[52] In addition, the microbiome of patients with ileal CD showed an increase in fungi at the expense of bacteria, whereas patients with UC and those with CD without ileal involvement exhibited reduced fungal diversity.[47]

ROLE OF GUT MICROBIOME IN IBD DIAGNOSIS

Recently, researchers have explored different approaches, such as machine learning (ML) methods to target specific microbiome signatures either the bacteria genes or species for disease classification.[53,54] To further support the impact of the gut microbiome in patients with IBD, recent studies have demonstrated the role of specific microbes in driving or suppressing inflammation, predicting response to therapy and determining the risk of recurrence after surgery. Herein we aimed to highlight the specific role of bacterial species and discuss the potential of utilizing these microbes as biomarkers for IBD diagnosis and prognosis (Table 1).

Disease diagnosis

Specific microbial signatures can be used to diagnose IBD especially if they were consistently present in higher levels in cases than in controls, and they may also have a role in differentiating CD from UC. *Faecalibacterium prausnitzii* is commonly found to be depleted in patients with IBD. Lopez-Siles et al. measured the abundance of *F. prausnitzii* and its two Phylogroups (I and II) in the intestinal mucosa of patients with IBD and controls using quantitative polymerase

TABLE 1 Gut microbial biomarkers for IBD diagnosis, classification and activity assessment

Study	Population	Sample size	Platform	Sample type	Marker	Purpose	Performance
Lopez-Siles et al. (2016)	Spanish	45 CD, 25 UC, 31 controls	qPCR	Biopsy	*Faecalibacterium prausnitzii* phylogroup I	Diagnose CD Diagnose UC	AUC = 0.851 AUC = 0.763
Guo et al. (2019)	Chinese	176 IBD (95 CD, 81 UC), 65 IBS, 105 controls	qPCR	Stool	Fecal markers *Fusobacterium nucleatum* (Fn) *F. prausnitzii* (Fp)	Distinguish CD from controls Distinguish CD from IBS	Fn: AUC = 0.841 Fp: AUC = 0.811 Fn + Fp: AUC = 0.867 Fn: AUC = 0.767 Fp: AUC = 0.658 Fn + Fp: AUC = 0.771
Pascal et al. (2017)	European (Spain, Belgium, UK and Germany)	Discovery cohort: 34 HC, 33 UC, 34 CD Validation cohort: 1247 HC, 339 CD, 158 UC, 202 IBS and 99 anorexia	16S sequencing	Stool	8 genera: *Faecalibacterium*, an unknown *Peptostreptococcaceae*, *Anaerostipes*, *Methanobrevibacter*, an unknown *Christensenellaceae*, *Collinsella* and *Fusobacterium*, *Escherichia*	Discriminate CD from UC, IBS, anorexia, and healthy control	Sensitivity: 80% Specificity: 90%–95%
Chamorro et al. (2021)	Chilean and Spanish	Chilean: 20 UC, 21 CD, 5 controls Spanish: 13 CD; 7 controls	16S sequencing	Biopsy	20 OPU (operational phylogenetic unit) 11 OPU	Discriminate dysbiosis IBD from eubiosis IBD (similar to controls) Discriminate UC from CD	AUC = 0.96–0.99 AUC = 0.83
Manandhar et al. (2021)	America	729 IBD, 700 non-IBD 331 CD, 141 UC	16S sequencing	Stool	50 bacterial taxa 117 bacterial taxa	Diagnose IBD Discriminate UC from CD	AUC = 0.80 AUC >0.90
Clooney et al. (2021)	Irish and Canadian	303 CD, 228 UC, 161 controls	16S sequencing	Stool	715 species 732 species	Discriminate CD from controls Discriminate UC from controls	AUC = 0.88 AUC = 0.88
Papa et al. (2012)	America	Discovery cohort: 23 CD, 43 UC, 1 undefined IBD, 24 non-IBD controls (pediatric patients)	16S sequencing	Stool	Selected features	Diagnose IBD	Discovery cohort: AUC = 0.83 (80.3% sensitivity and 69.7% specificity) Validation cohort: AUC = 0.84 (92% sensitivity, 58.5%

TABLE 1 (Continued)

Study	Population	Sample size	Platform	Sample type	Marker	Purpose	Performance
		Validation cohort: 25 CD, 30 UC, 13 controls (children and young adults)					specificity)
						Discriminate UC from CD	AUC = 0.76
						Assess the activity of IBD	AUC = 0.72
Gevers et al. (2014)	North America	447 pediatric CD, and 221 control subjects (RISK)	16S sequencing	Biopsy and stool	Microbial dysbiosis index (MD-index)	Diagnose CD	Terminal ileum biopsies: AUC = 0.85
							Rectum biopsies: AUC = 0.78
							Stool: AUC = 0.66
Franzosa et al. (2019)	USA (PRISM)	68 CD, 53 UC, 34 controls	Metagenomics	Stool	Microbial species	Diagnose IBD	AUC = 0.90
							AUC = 0.86
	Netherlands	20 CD, 23 UC, 22 controls	LC-MS		Metabolites		AUC = 0.92
							AUC = 0.89
Serrano-Gomezal et al. (2021)	America, Spanish, Belgian	65 CD, 38 UC, 27 controls (America); 34 CD, 33 UC, 67 controls (Spanish); 49 CD (Belgian)	Metagenomics	Stool	26 species	Diagnose CD	AUC = 0.938
					9 species	Diagnose UC	AUC = 0.646
					11 species	Predict CD relapse	AUC = 0.769
Zuo et al. (2022)	Caucasian	7 pediatric UC, 8 controls	16S sequencing	Stool	Genus	Diagnose UC	AUC = 0.869
			Metagenomics		Species		AUC = 0.763
			Metagenomics		Pathway		AUC = 0.764
Kolho et al. (2015)	Finland	68 pediatric IBD, 26 controls	Phylogenetic microarray	Stool	9 bacterial groups	Assess the activity of IBD	AUC = 0.85
Tedjo et al. (2016)	Netherlands	71 CD (97 active and 97 remission samples)	16S sequencing	Stool	50 OTUs	Assess the activity of CD	AUC = 0.82
El Mouzan et al. (2018)	Saudi Arabia	15 pediatric CD, 20 controls	ITS sequencing	Stool	Selected features	Diagnose CD	AUC = 0.85
				Biopsy			AUC = 0.71
Sarrabayrouse et al. (2021)	Spanish and Belgian	65 IBD, 28 controls	qPCR	Stool	Microbial load data + demographic and standard laboratory data	Diagnose IBD	AUC = 0.842

Abbreviations: AUC, area under the curve; CD, Crohn's disease; HC, healthy control; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; ITS, internal transcribed spacer; LC-MS, liquid chromatograph mass spectrometer; OPU, operational phylogenetic unit; qPCR, quantitative polymerase chain reaction; UC, ulcerative colitis.

chain reaction (qPCR) assay. They found that *F. prausnitzii* phylogroup I had the best performance in discriminating healthy subjects from subjects with CD (area under the curve [AUC] = 0.851) and UC (AUC = 0.763).[55] In a Chinese cohort, the presence of *F. prausnitzii* and *F. nucleatum* in stool samples based on qPCR assay showed a good performance with an AUC of 0.841 and 0.811, respectively.[56] In a study of 2045 non-IBD and IBD patients from four European countries, an algorithm based on eight selected genera from stool samples (*Faecalibacterium*, an unknown *Peptostreptococcaceae*, *Anaerostipes*, *Methanobrevibacter*, an unknown *Christensenellaceae*, *Collinsella* and *Fusobacterium*, *Escherichia*) performed well in discriminating CD from UC, irritable bowel syndrome, anorexia, and healthy control.[57] By assessing 20 bacteria markers in mucosal samples from Chilean and Spanish patients with IBD, Chamorro et al.[58] were able to discriminate dysbiosis and eubiosis in IBD patients, with an AUC ranging from 0.96 to 0.99.

Others have selected more bacterial markers as biomarkers for IBD determination using various feature selection methods and ML models.[59–61] Manandhar et al.[53] selected 50 fecal bacterial taxa for disease diagnosis in a large American cohort. By using five different supervised ML algorithms, their classifiers attained an AUC of around 0.80. A recent Irish and Canadian study[62] identified a large number of species from subjects' stool samples for differentiating IBD subtype and controls, achieving an AUC of 0.88 for separating UC and CD from controls. Among them, *Eubacteria rectale* and *Clostridium* cluster XIVa, which were both decreased in CD and UC patients, were the most contributing operational taxonomic units (OTUs) in disease diagnostic models. In another large pediatric cohort, Gevers et al.[63] calculated the microbial dysbiosis index (MD-index) for early diagnosis of CD using biopsies and stool samples, showing the best performance in terminal ileum biopsies and marginally worse results in rectum biopsies.

Metagenomic sequencing, which has a higher taxonomy resolution that enables better identification of specific bacterial species or strains related to disease development, is increasingly applied for the discovery of microbial markers. Franzosa et al.[64] trained a random forest classifier on selected fecal microbial species and found a consistent diagnostic accuracy with AUC of 0.90 in the discovery cohort from USA and AUC of 0.86 in a validation cohort from the Netherlands. Serrano-Gomez et al.[65] used the 26 species and nine species for predicting CD with an AUC of 0.938 and predicting UC with an AUC = 0.646. Notably, *Veillonella parvula*, *E. coli*, *R. gnavus and Clostridium clostridioforme* were significantly enriched species in CD compared with UC and healthy controls, while *F. prausnitzii* is the most depleted species in CD. Interestingly, Zuo et al.[44] demonstrated that although the 16s data shows similar results as shotgun sequencing data in terms of alpha diversity and beta diversity, 16S genus data (AUC = 0.869) achieved higher pediatric UC prediction performance than shotgun species data (AUC = 0.763) and pathway data (AUC = 0.764).

Alterations in the gut mycobiome have also been explored for disease diagnosis and ecological processes.[66] El Mouzan et al.[67] reported fungal dysbiosis in mucosa and stool of patients with CD and found that the performance of the classifier based on stool samples was significantly higher (AUC = 0.85) than that of mucosal samples (AUC = 0.71). Sarrabayrouse et al.[68] examined the role of fungal and bacterial loads in predicting IBD, IBD subtypes, and disease flare. They showed that combined with the demographic and standard laboratory data, and microbial load data from stool samples improved the performance of the random forest models for IBD diagnosis (AUC = 0.842). However, the potential role of fungal and viral dysbiosis in the diagnosis of IBD have not been studied extensively, due to the low abundance of fungal and viral DNA relative to bacterial DNA and the limited available genome references. But with the development of detection method and the improvement of fungal and viral database, gut mycobiome and virome will be completely captured and explored. The disease diagnostic performance will be further enhanced by combining the new-found fungal and viral biomarkers with the existing bacterial biomarkers.

However, most of these studies were based on the sequencing results. Although the NGS technologies facilitate the microbiome analysis by providing the composition and relative abundance of microbes, they also have disadvantages, including high cost, complicated operations, sophisticated results interpretation, and low detection sensitivity. The use of qPCR assays for microbial markers to screen colorectal cancer provides a new direction for disease diagnosis and management.[69] qPCR detection is a cheap, easy-to-use, and multiplexed technique. Therefore, the development of qPCR-based microbial marker detection methods or other affordable methods[70] to realize the diagnosis and prognosis of IBD has great potentials. This will simultaneously lead to the problem that the numbers of microbiome markers used for IBD diagnosis and prognosis should be balanced with the diagnosis and prediction performance. Selecting several most contributing biomarkers for detection would be more cost-effective.

Besides, the above studies mostly based on the cohorts from Europe and North America, only few Asian cohorts were included. This may be attributed to the different incidence rate of IBD in difference region. Studies have revealed that the geographical, diet and lifestyle variations have a great impact on the human microbiome. In this regard, it is important to find out the similarities and differences in gut microbial variations between people of different races. Universal and region-specific microbial markers should be developed to achieve a more accurate diagnosis performance.

Overall, the development of microbial biomarkers for disease prediction and diagnosis appeared promising and may complement current more invasive diagnostic modalities. However, further validation of each marker or a combination of markers needs to be performed in different populations and inter-individual variations of microbial markers should be studied.

Disease classification

Although symptoms of CD and UC are relatively similar, their treatment, outcomes and need for surgery differ. Thus, it is necessary

to differentiate the subtypes of IBD accurately. Studies have used fecal or mucosal bacteria signatures to distinguish UC from CD. Manandhar et al.[53] identified 117 differential bacterial taxa from stool samples for discriminating CD with UC which showed excellent performance (AUC >0.90). Using only 11 mucosal bacteria, Chamorro et al.[58] could differentiate UC from CD with an AUC of 0.83. These findings suggest the possibility of using a specific set of microbes for IBD subtype classification. However, a recent study that included Irish and Canadian patients with IBD showed a lower AUC value of 0.60–0.70 in differentiating CD from UC. Interestingly, the most important OTU in this model was *F. prausnitzii*.[62]

Disease activity

Since IBD is a chronic disease with long-term therapeutic strategies, a better non-invasive tool for disease assessment is needed. Currently, fecal calprotectin is commonly used, but levels can be raised in any inflammatory conditions and may not be specific to IBD.[71–73] Effective and better monitoring of disease activity can help clinicians to assess the disease status, and tailor treatment more efficiently. Association between bacterial markers and disease severity scores have raised the possibility of using them as indicators of disease status.[74,75] Kolho et al.[76] performed a phylogenetic microarray with 9 bacterial groups to assess the activity of IBD, leading to an AUC of 0.85 when using 100 μg/g as the cutoff of fecal calprotectin levels. Tedjo et al.[75] also identified a discriminatory panel of fecal microbes to differentiate between active and inactive CD with an AUC of 0.82. These data highlight the potential of fecal microbial signatures in monitoring disease activity.

ROLE OF GUT MICROBIOME IN DISEASE PROGNOSIS AND RESPONSE TO THERAPY

Disease recurrence

IBD comes with natural courses following with periods of remission and relapse. Studies have showed that the gut microbiome dysbiosis may be involved in the disease activity of IBD.[77] A systematic review showed that *C. leptum*, *F. prausnitzii* and *Bifidobacterium* decreased in CD and UC patients with active disease status when compared to patients in remission. But lower abundance of *Clostridium coccoides* was only found in active UC patients but not CD patients.[78] *Streptococcus* levels were also found to be more abundant in samples of patients with postoperative CD recurrence.[79] The profile of mucosa-related gut microbiota in the ileum of patients with CD exhibited significant alterations following surgery. Sokol et al.[80] reported that reduction in alpha diversity and an increase in the *Proteobacteria* phylum were linked to endoscopic recurrence of CD, with an accompanying decrease of members from *Lachnospiraceae* and the *Ruminococcaceae* families within the *Firmicutes* phylum. The alteration of gut microbiota at the time of surgery can predict endoscopic recurrence with the AUC of 0.81. The most contributing taxa in this model were *Streptococcus*, *R. gnavus* group and *Gammaproteobacteria*. Patients with CD who had recurrence after surgery had elevated *Proteus* genera and reduced *Faecalibacterium* in their mucosa, and recurrence was also associated with a history of smoking.[81] Moreover, in this study, a model comprising the *Proteus* genera, the abundance of *Faecalibacterium*, and smoking status has been developed to predict postoperative CD recurrence and showed moderate accuracy (AUC = 0.740).[81] Machiels et al.[82] showed there were a different mucosal microbiome after ileocecal resection between recurrence and non-recurrence CD patients. *Fusobacteria* was thought to be the most prominent player driving early postoperative disease recurrence. Based on the mucosa-associated genera *Ralstonia*, *Haemophilus*, *Gemella*, and *Phasolarctobacterium* at the time of resection, the AUC of predicting postoperative endoscopic recurrence was 0.739. And the AUC is 0.875 when using the fecal microbiome (*Coprobacilus*, unidentified *Lachnospiraceae* genus, and *Dorea*) at the time of resection. Serrano-Gomez et al.[65] used 11 species based on stool metagenomic data for predicting CD relapse with the AUC of 0.769. *R. torques*, *Fusicatenibacter saccharivorans*, *Clostridium bolteae* were the significant differentially abundant species between CD remission and relapse.

Therapeutic response

The gut microbiota and specific bacteria taxonomic features have also been shown to influence drug response and outcome.[83–86] In a prospective study of serial fecal samplings of patients with CD who were initiating anti-integrin inhibitors, Ananthakrishnan et al.[87] found that patients achieving remission had a higher α-diversity and higher abundance of *Roseburia inulinivorans* and *Burkholderiales* species at baseline, as well as branched-chain amino acid biosynthesis pathways compared to those who did not achieve remission, and the predictive ability using the microbial taxa (AUC = 0.715) performed better than utilizing clinical data alone (AUC = 0.619) in predicting remission.

Zhou et al.[77] showed that microbial taxonomy, mainly *Clostridiales*, have great high prediction ability in predicting response to infliximab treatment with 86.5% accuracy alone and with 93.8% accuracy when combined with calprotecin levels and CDAI. A multi-omics study combining fecal metagenomic, serum metabolomic, and proteomic markers has identified several markers that predict differential response to IBD biologic therapy.[88] The performance of model using clinical and metagenomic features in classifying remission at week 14 among patients taking anti-cytokine therapy was higher (AUC = 0.849) than using the clinical variables only (AUC = 0.624). The abundances of nine bacterial species at baseline were correlated to earlier remission, among which included *Phascolarctobacteriaum faecium*, *Agathobaculum butyriciproducens*, and *Clostridium citroneae*, and these bacteria have been previously reported to have anti-inflammatory effects.[89] Following validation, six of the nine species markers were linked to anti-cytokine response. Interestingly, a study analyzed the gut mycobiota and found that *C. albicans* was

more abundant in non-responders than responders to the anti-TNF agent, Infliximab.[90] In a systematic review of 19 studies, increased baseline gut bacteria α-diversity was observed in subjects with IBD who achieved response with exclusive enteral nutrition, Infliximab, Ustekinumab or Vedolizumab. Moreover, an increase in the abundance of *F. prausnitzii* was noted in subjects who responded to aminosalicylates, anti-TNF medications and Ustekinumab.[91] Overall, these data support the importance of gut microbiome composition in determining treatment response in IBD.

ROLE OF MICROBIAL-DERIVED METABOLITES IN IBD

The gut microbiota can produce a variety of bioactive metabolites that can be absorbed into the enterohepatic circulation and then into the host circulatory system.[92] These bacteria metabolites and derivatives affect host energy homeostasis, inflammation, endocrine regulation, and regulate host metabolism. Metagenomic analysis, plus targeted and untargeted metabolomics analysis have been utilized to investigate the function of microbiota-derived metabolites and the importance and variation of metabolites from feces, urine, and serum between IBD patients and healthy controls.[93,94]

Some bacterial-associated metabolites, including short-chain fatty acids,[64] medium-chain fatty acids,[95] tryptophan,[96,97] bile acid[98] and sphingolipid,[99] have shown great potential as new biomarkers in IBD diagnosis and prediction. Marchesi et al.[100] first characterized the fecal extracts from CD and UC patients and found a decrease in butyrate, acetate, methylamine, and trimethylamine, as well as an increase in amino acids. Based on principal component analysis, they showed distinct clustering that separated CD patients from healthy controls, and UC patients from healthy controls, suggesting that metabolite profiling may help discriminate patients with IBD from healthy individuals. In an Italian cohort of IBD patients, biogenic amines, amino acids and lipids were significantly increased in their stool, while two B group vitamins were decreased compared to healthy subjects.[101] orthogonal partial least square-discriminant analysis could separate both CD patients from healthy controls and UC patients from healthy controls.[101] In a study of 155 subjects from the USA cohort and 65 subjects from Netherlands, metabolomics analysis showed that eight fecal metabolites were significantly increased in CD patients compared with controls, the most prominent of which were sphingolipids, carboximidic acids and bile acids. Compared with the control group, the levels of seven metabolites were significantly increased in fecal samples of UC patients, while the level of phenylacetamides was increased, but the difference was not statistically significant. In addition, levels of metabolites such as chenodeoxycholate, C22:0-sphingomyelin, 2-hydroxymyristic acid, C54:6 TAG, lactate and pantothenate were significantly different between CD, UC and controls. The classifiers based on selected metabolites features achieved high accuracy (AUC = 0.92 in USA cohort, AUC = 0.89 in Netherlands cohort) in predicting IBD.[64]

ROLE OF MICROBIOTA-ASSOCIATED PROTEINS IN IBD

Meta-proteomic studies, a vital complement of metagenomics, offer new possibilities for the characterization of proteins from host or microbes associated with IBD and understanding the functional roles and interactions of microbes in communities.[102–105] With the use of untargeted and targeted proteomics, 12 bacterial proteins and one human protein were found to be over-represented or under-represented in patients with CD compared with healthy subjects.[106] In another study,[107] seven serum proteins analyzed by enzyme-linked immunosorbent assay (ELISA) could easily distinguish controls from patients with IBD with an AUC of 0.785. When additional serum biomarkers of the gut barrier, such as matrix metalloproteinase (MMP)-9, MMP-14, and tissue inhibitor of metalloproteinases 1 were included in the test set, the AUC increased to 0.904. MMP-9 and MMP-14 were also important contributors in the model for discriminating UC patients from controls, and CD patients from controls. In classifying CD and UC, the model using a set of selected proteins showed good performance with an AUC of 0.9. A recent study[108] also reported that stool proteins identified by an aptamer-based screen and validated by ELISA could distinguish patients with UC from controls or patients with CD from controls. Some of these proteomic stool biomarkers showed a stronger correlation with the disease activity in patients with UC who were followed up longitudinally. Their performance in disease monitoring and prediction was also higher when compared with fecal calprotectin alone. However, studies that have identified protein markers were mainly based on a very small sample size, and subtle differences between the disease and healthy group have been ignored. Hence, large cohorts are required to explore useful, reproducible and reliable protein markers for IBD prediction and prognosis in the future.

CONCLUSION

In summary, over the past few decades, an increasing number of animal and human studies have shown consistent alterations in gut microbiome composition that contributes to IBD pathogenesis. Emerging data have focused not only on the gut bacteria taxa but also on other microbial communities including the fungi, viruses, microbial metabolites and microbe-associated proteins. These data have shed light on the potential role of microbiome biomarkers in IBD diagnosis and prediction. Recent advances in sequencing technology and analytical platforms with validation across different disease phenotypes and populations will improve our understanding of perturbations of the microbiome-metabolome interface in IBD, as well as drive further identification of potential diagnostic markers and therapeutic targets.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

No data was created in this review.

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How to cite this article: Zheng J, Sun Q, Zhang J, Ng SC. The role of gut microbiome in inflammatory bowel disease diagnosis and prognosis. United European Gastroenterol J. 2022;10(10):1091–102. https://doi.org/10.1002/ueg2.12338