Microbiome-Based Biomarkers for IBD

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Crohn disease and ulcerative colitis are complex immune-mediated diseases that are characterized by a heterogeneity in presentation and clinical course. Although various clinical covariates predict adverse outcomes in these patients, they are insufficiently informative. The gut microbiome likely plays a central role in the pathogenesis of these diseases. Consequently, microbiome-based biomarkers may play an important role in risk stratification and disease prediction. Initial cross-sectional studies showed a reduced gut microbial diversity in patients with Crohn disease or ulcerative colitis, a depletion of phyla with anti-inflammatory effects such as those belonging to *Firmicutes,* and an increased abundance of potentially pathogenic bacteria in specific disease phenotypes. Subsequent studies longitudinally tracking microbial changes and clinical outcomes have shown dynamic changes correlating with or predictive of disease activity and resistance to therapy. The development of multicenter cohorts using harmonized protocols is essential to robustly validate these biomarkers and facilitate the integration of their evaluation into clinical practice.

Key Words: Crohn disease, ulcerative colitis, microbiome, biomarker

BACKGROUND

Crohn disease (CD) and ulcerative colitis (UC), together constituting inflammatory bowel diseases (IBD), affect more than 2 million individuals in the United States and many more worldwide.[1] The prevailing view on their pathogenesis suggests that they arise from a dysregulated immune response to an altered gut microbiome with a background of genetic predisposition.[2-5] Findings over recent years have highlighted the central role of the gut microbiome in the pathogenesis of these diseases.[6-12] Studies more than 2 decades ago demonstrated that exposure to fecal luminal contents is important to establish intestinal inflammation.[13] The lesions of CD can be resolved by proximally diverting the feces away from a segment of the intestine, and they recur upon re-exposure to fecal contents.

The next wave of microbiome studies, using predominantly 16s rRNA sequencing methods, showed the dissimilarity between the microbiome in patients with IBD and that of healthy control patients.[6-12] There is a reduced diversity in those patients with established disease who also show depletion of key phyla that are important for their production of anti-inflammatory metabolites such as butyrate.[7, 12] Many of the species depleted in those with IBD are within the genus *Firmicutes.*[11, 12, 14, 15] In addition, although studies have broadly been unable to define a single or group of pathogenic organisms responsible for intestinal inflammation in all patients, the increased abundance of specific species (such as adherent invasive *Escherichia coli*) may contribute to specific disease phenotypes (ileal CD).[16] These findings have been consistently replicated in various populations across a spectrum of clinical settings. Multiple comprehensive reviews have highlighted the differences in the compositional and functional aspects of the microbiome in IBD compared with one in a healthy state,[8, 9, 17-19] so those differences are not further discussed in this review.

The second wave of microbiome studies focused on a longitudinal rather than a cross-sectional examination of the microbiome; they offered intriguing evidence of transient blooms of specific genera, species, or strains that may contribute to inflammation through production of inflammatory mediators (such as an inflammatory polysaccharide production by *Ruminococcus gnavus*).[11, 14, 20-23] These studies have also allowed examination of the predictive utility of the microbiome (and consequently microbial biomarkers) in identifying those at risk for adverse clinical outcomes including relapse, therapy failure, or disease progression.

Given that existing clinical parameters perform poorly in predicting each of the above outcomes, it is in this space that the use of microbial biomarkers offers much promise. Particularly, as outlined in this article, through the utilization of sophisticated machine learning–based predictive models, several studies have shown that such microbiome-based biomarkers outperform clinical parameters in predicting these outcomes.[24-27] In this review, I highlight recent key studies that illustrate the utility of profiling the microbiome to predict 3 clinically relevant outcomes in IBD and conclude by suggesting future avenues of research that overcome the limitations of existing data (Fig. 1).

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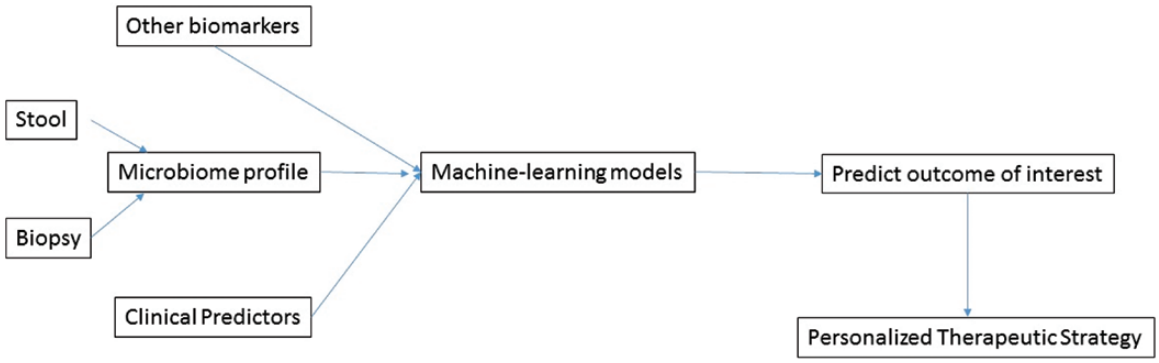

FIGURE 1. Conceptual framework for incorporation of microbiome-based biomarkers in the management of IBD.

Tools for Microbiome Sequencing

There are several recent reviews on sequencing and analysis of the microbiome, although a full discussion of these methods is beyond the scope of this review.[28-31] In brief, commonly used methods to study the microbiome use either amplicon sequencing methods that analyze the partial genome or whole-genome shotgun metagenomic sequencing. Amplicon sequencing methods profile segments of the bacterial genome, typically the hypervariable regions of the highly conserved 16s ribosomal RNA gene. Such profiling can reveal segments (operational taxonomic units [OTUs]) that are common to a specific taxon. A limitation of this method is that closely related bacterial families may share 99% of the hypervariable region, thereby precluding identification of individual species and strains. A second commonly used microbial sequencing method relies on shotgun metagenomic sequencing, which is high-throughput sequencing of the entire bacterial genome. Through profiling the entire genome, this method allows for resolution at the species and even strain level in addition to the definition of bacterial function through mapping the genome to reference databases.

Analysis of both 16s rRNA and metagenomic sequencing has traditionally relied on comparing the relative abundance of various genera, species, and strains between different groups. A limitation of this method is that a change in relative abundance could result from either the increased absolute number of a species of interest or the depletion of other species and consequent reliance on the overall microbial load. In contrast, quantitative profiling of absolute counts of absolute microbial abundances may be as or more relevant to defining the role of microbiome in human disease.[32] A small study of 29 patients with CD revealed microbial counts that were 3 times lower than those of healthy control patients and differences in defining an association between *Bacteroides* (noted only in relative profiling) and *Prevotella* (only in quantitative microbial profiling) in CD.[32]

Prediction of Relapse and Postoperative Recurrence

One of the first studies to examine the role of the microbiome in relapse in IBD was by Rajca et al[33] (Table 1). The investigators obtained fecal samples from 33 patients with CD who stopped infliximab therapy (as part of an interventional study to assess the rate of relapse after biologic cessation). Fecal samples were obtained at therapy cessation, 2 months, 6 months, and the end of follow-up. Cross-sectional comparisons were also made with 29 healthy control patients. Over follow-up, 19 patients relapsed and 14 remained in remission. Compared with the healthy control patients, those with CD had reduced counts of *Firmicutes* such as *Clostridium coccoides* (P = 0.0003), *Clostridium leptum,* and *Faecalibacterium prauznitzii* (P = 0.003). Interestingly, although all patients were in clinical remission at study entry, a lower baseline abundance of *F. prausnitzii* (P = 0.014) and *Bacteroides* (P = 0.030) predicted relapse after therapy cessation in addition to biochemical markers such as an elevated serum C-reactive protein.

The association with *F. prauznitzii* is particularly interesting as prior work has shown a significant anti-inflammatory effect of this species. In experiments using in vitro and in vivo models, Sokol et al[34] showed in 2008 that stimulation of peripheral blood mononuclear cells by *F. prauznitzii* resulted in lower interleukin (IL)-12 and interferon-γ levels and higher levels of IL-10, which has known anti-inflammatory effects. Interestingly, the oral administration of *F. prauznitzii* resulted in a lower severity of 2,4,6-trinitrobenzenesulfonic acid colitis. Note that the culture supernatant also had a similar effect in reducing the severity of this particular colitis, suggesting that the effect of *F. prauznitzii* may be mediated in part through the production of anti-inflammatory metabolites. A subsequent study of a peptidomic analysis of *F. prauznitzii* culture supernatants showed 7 peptides derived from a microbial anti-inflammatory molecule that had a dose-dependent effect on reducing the activation of the NF-κB pathway.[35] Luminal delivery of this peptide through *Lactococcus lactis* alleviated colitis in dinitrobenzene sulfonic acid mice models.[35] In other experiments, *F. prauznitzii* or its supernatant also induced the production of IL-10 and transforming growth factor-β1.[36]

Most studies that have examined the ability of the microbiome to predict future disease activity from a state of quiescence have used the postoperative model in CD. A small study of 12 patients with CD examined the microbiota profile

TABLE 1. Summary of Studies of the Microbiome in Predicting Disease Outcomes in IBD

Author	Year	Patient Population	Microbiome Source and Profile	Outcome of Interest	Main Findings
Disease relapse					
Rajca et al[33]	2014	33 patients with CD in remission	Stool, qPCR	Clinical relapse	Relapsers had lower abundance of *F. prausnitzii* ($P = 0.014$) and *Bacteroides* ($P = 0.030$) than nonrelapsers
De Cruz et al[37]	2015	12 patients with CD undergoing ileal resection	Ileal biopsy, 454 pyrosequencing	Postoperative recurrence	Lower abundance of *Clostridiales* and *Bacteroidales* orders in those with later recurrence and a greater representation of *Bacilli* order (especially *Streptococcaceae and Enterococcaceae*), *Enterobacteriaceae,* and *Veillonellaceae*
Sokol et al[38]	2020	201 patients with CD undergoing ileal resection	Ileal biopsy, 16s sequencing	Postoperative recurrence	*Gammaproteobacteria, Corynebacterium, Ruminiclostridium,* and *R. gnavus* group; others associated with endoscopic recurrence at 6 months
Treatment response					
Michail et al[44]	2012	27 children with acute severe UC initiating steroid therapy	Stool, 15s sequencing	Steroid response	Steroid responders had higher baseline diversity than nonresponders
Kolho et al[41]	2015	11 children with IBD initiating anti-TNF therapy	Stool, qPCR	Response: reduction in fecal calprotectin	Responders had higher baseline levels of *Bifidobacterium, C. colinum, E. rectale,* uncultured *Clostridiales,* and *Vibrio* and lower abundance of *S. mitis*
Lewis et al[10]	2015	90 children initiating anti-TNF therapy or enteral nutrition	Stool, metagenomics	Reduction in fecal calprotectin at week 8	*Lactococcus* and *Roseburia* abundance increased with resolution of inflammation at week 8 and *Actinomyces* abundance decreased
Magnusson et al[43]	2016	56 patients with UC initiating anti-TNF therapy	Stool, rtPCR, and GA-map; dysbiosis test	Clinical response at 12-14 weeks	Lower dysbiosis index and higher abundance of *F. prausnitzii* at baseline in responders than in nonresponders
Ananthakrishnan et al[25]	2017	85 patients with IBD initiating vedolizumab	Stool, metagenomics	Clinical response at week 14	Higher baseline alpha diversity (species) in responders than in nonresponders (in CD); greater abundance of *R. inulinivorans* and *Burkholderiales* at baseline in responders; several functional differences on pathway analysis
Doherty et al[27]	2018	232 patients with CD initiating ustekinumab	Stool, 16s sequencing	Clinical remission at week 6	Two OTUs affiliated with *Faecalibacterium* and *Bacteroides* were more abundant at baseline in those with remission at week 6
Zhou et al[26]	2018	16 patients initiating infliximab	Stool, 16s sequencing	Clinical response at week 30	Abundance of *Clostridiales, Veillonella, Bacteroides,* and *Anaerostipes* at baseline predicted response
Aden et al[24]	2019	12 patients with IBD initiating anti-TNF therapy; validation cohort of 23 patients	Stool, 16s sequencing	Clinical response at week 14	None of the taxa predicted response to anti-TNF therapy. However, differences in metabolite exchange noted including less-frequent exchange of butyrate, L-arginine, ammonium, ornithine, ethanol, L-glutamine, and glycine.
Ribaldone et al[42]	2019	20 patients with CD initiating adalimumab	Stool, metagenomics	Change in microbiome with clinical response	*Proteobacteria* decreased in patients in whom therapeutic success was obtained but not in nonresponders
Disease progression					
Kugathasan et al[14]	2017	913 children newly diagnosed with CD	Stool, ileal and rectal biopsy, 16s sequencing	Development of stricturing or penetrating disease	Abundance of *Ruminococcus* and *Rothia* associated with development of stricturing disease and *Collinsella* and *Veillonella* abundance predicted penetrating complications
Hyams et al[23]	2019	400 children newly diagnosed with UC	Stool, rectal biopsy, 16s sequencing	Corticosteroid-free remission at week 52	Abundance of *Ruminococcaceae* and *Sutterella* at baseline predicted week 52 remission

Abbreviations: GA-map, genetic analysis; qPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction.

from ileal tissue from CD patients undergoing resection.[37] In comparison with healthy control patients, the patients with CD had a lower biodiversity at the time of the surgery that improved postoperatively but was still less diverse than that of the healthy control patients. Note that patients with CD who had endoscopic recurrence had a greater abundance of *Enterococcus* and *Veillonella* and that those who remained in postsurgical remission had a higher abundance of butyrate-producing *Firmicutes, Bacteroides, Prevotella,* and *Parabacteroides*. A more robust examination of this result was in the large REMIND study, a French cohort of 201 patients with CD undergoing an ileocecal resection.[38] Here the authors examined the mucosa-associated microbiota using 16s rRNA sequencing. At month 6, several phyla were reduced in patients who had endoscopic recurrence (many belonging to *Firmicutes,* including *Ruminococcaceae* and *Lachnospiraeceae*), and there was an expansion of *Proteobacteria.* On multivariable analysis, the presence of 6 microbial phyla (*Gammaproteobacteria, Corynebacterium, Sellimonas, Ruminiclostridium, Hydrocarboniphaga,* and *R. gnavus)* predicted endoscopic recurrence. However, the predictive value was significantly greater among those patients who did not receive anti-tumor necrosis factor (anti-TNF) therapy postoperatively than among those who did, suggesting that ongoing treatment may modify natural history and reduce the predictive utility of microbial markers.

Response to Biologic and Nonbiologic Therapies

Several studies have examined the utility of microbial markers in predicting relapse in patients with CD or UC.[39-41] An initial analysis of the longitudinal changes in the microbiome was conducted by Kolho et al,[41] who profiled 68 children with IBD among whom 32 received anti-TNF therapy. During induction, patients who responded (defined by a reduction in fecal calprotectin) showed increasing diversity and similarity to healthy control patients, whereas a similar effect was not observed among those who were nonresponders. Although the abundance of *Bacilli, Proteobacteria,* and low-abundance *Clostridium* clusters increased in nonresponders, these levels did not increase in responders. The authors showed that patients responding to anti-TNF therapy had higher baseline abundances of bacteria belonging to *Bifidobacterium, Clostridium colinum, Vibrio,* and *Eubacterium rectale* and a lower abundance of *Streptococcus mitis.* A multivariable model that incorporated an abundance of *Clostridium sphenoides* and *Haemophilus* species predicted a fecal calprotectin level < 200 mcg/g at 3 months with an accuracy of 0.88.[41]

Lewis et al[10] performed metagenomics sequencing on stool samples from 86 pediatric patients who were initiating therapy with either exclusive enteral nutrition, anti-TNF therapy, or partial enteral nutrition therapy. Comparisons were performed between stool samples obtained at baseline, 1 week, 4 weeks, and 8 weeks after therapy and with 26 previously profiled healthy children. When compared with healthy children, patients with CD differed in the median abundance of 14 (out of 45) general bacteria, including reductions in *Prevotella, Akkermansia, Roseburia, Alistipes, Coprococcus,* and *Ruminococcus* and an increased abundance of various members of *Enterobacteriaeceae* including *Escherichia* and *Klebsiella.* At the end of the study, responders' microbial composition was closer to that of healthy control patients than nonresponders' microbial composition, an effect that was also separately observed for anti-TNF therapy and exclusive enteral nutrition. Among the patients on anti-TNF therapy, 11 taxa differed from those of healthy control patients at baseline and 6 of these remained significantly different despite clinical response. Response to therapy (defined as a fecal calprotectin level < 250 mcg/g) was associated with a decrease in *Actinomyces* and an increase in *Lactococcus* and *Roseburia.* In contrast, response to therapy was not associated with a change in fungal composition. Ribaldone et al[42] prospectively studied the fecal microbiome in 20 patients with CD initiating adalimumab therapy. In those who responded to treatment, there was a decrease in *Proteobacteria* and an increase in *Lachnospiraceae.*

Magnusson et al[43] profiled antimicrobial peptide expression from both mucosal biopsies and the fecal microbiome in 56 patients with CD and 7 patients with UC who were anti-TNF-naïve and were initiating biologic therapy with this class. Greater expression of antimicrobial peptides including DEF5, BPI, ECP, HMGB1, and HMGN2 was noted in responders but not in nonresponders. Consistent with other observations, responders had a less dysbiotic microbiome at baseline than nonresponders. The baseline abundance of *F. prauznitzii* was higher in responders than in nonresponders. The difference in the abundance of *F. prauznitzii* was more striking at later time points, at week 2 and week 6, when responders showed an increasing abundance from baseline and nonresponders had no such changes.[43] Up to one-third of patients with UC develop acute severe colitis. In a study by Michail and colleagues, patients who were steroid-responsive showed greater diversity at baseline than nonresponders.[44]

The author and colleagues previously performed metagenomic sequencing from fecal samples of 85 patients with IBD initiating treatment with vedolizumab.[25] The study cohort included 43 patients with UC and 42 patients with CD. The primary outcome, remission at week 14, was attained by 31 patients. A total of 35% of the cohort remained in remission at week 54. In the baseline stool sample, alpha diversity at the species level was higher among those with CD who achieved remission at week 14 than in those who were not in remission at that time point. This difference was noted primarily at the species level. Compositionally, there was no separation of responders and nonresponders on principal component analysis, primarily because of similar baseline abundance of the top 15 most abundant species. Two species, *Roseburia inulinivorans* and *Burkholderiales,* were significantly more abundant at baseline in CD patients who achieved remission than in those who

did not (q = 0.09 and 0.06, respectively). Strikingly, more differences were noted on functional pathways: 13 were enriched at baseline in patients who achieved remission at week 14, including branched chain amino acid biosynthesis, arginine, and polyamine biosynthesis. In patients who provided stool samples at baseline and weeks 14, 30, and 54, remission at week 14 was associated with greater microbial persistence, defined as the similarity of later samples to the week 14 microbial profile, up to 1 year. Baseline clinical data alone were insufficient in predicting remission at week 14 (area under the curve [AUC] = 0.62), but a neural network model incorporating taxonomic and pathway data improved predictive ability with an AUC of 0.872.

Zhou et al[26] followed 16 Han Chinese patients with CD who were treated with infliximab for 30 weeks. Treatment was associated with an increase in alpha diversity and reduction in disease activity, which was more notable in the responder group. They also observed that abundance of *Clostridiales* was lower in those with IBD than in control patients. However, this difference was no longer significant in those who responded, suggesting that an increase in abundance of *Clostridiales* could be a biomarker of treatment response. A model of gut microbial composition at baseline predicted infliximab response at week 30 with an 87% accuracy, which was superior to the predictive value of the Crohn's Disease Activity Index (58% accuracy) and calprotectin levels (62% accuracy). The most informative features in this model were the abundance of various *Clostridiales* and the abundance of *Veillonella, Bacteroides,* and *Anaerostipes.*

Aden et al[24] performed a prospective study of 12 patients with IBD, 17 patients with rheumatic disease, and 19 healthy control patients who provided fecal samples at baseline and 2, 6, and 30 weeks after treatment with anti-TNF therapy. Twenty-three patients who were treated with either anti-TNF drugs or vedolizumab acted as control patients. Interestingly, patients with IBD but not with rheumatic disease demonstrated a shift in diversity toward that of the control patients with anti-TNF therapy, suggesting that this effect was likely mediated in part by resolution of intestinal inflammation rather than direct effect of the biologic agent.[24] When compared to healthy control patients, 14 indicator phylotypes, notably *Coprococcus* and *R. inulinivorans,* were differentially distributed between patients with IBD and healthy control patients. All 14 species had a similar abundance to that of control patients at week 30 that increased although none of these changes were specific to responders.

Similar to the observations by Ananthakrishnan et al,[25] the predictive value of taxonomic composition was less striking than the bacterial metabolic effects. None of the taxa profiled predicted response to anti-TNF therapy. However, the rate of metabolite exchange between organisms (ie, metabolic interchange) was lower at baseline in nonresponding patients with IBD than in healthy control patients in contrast to those who achieved remission, in whom this interchange was similar to that in healthy control patients. Specifically, the exchange of 10 metabolites was less frequent at baseline among patients who did not achieve remission compared with healthy control patients; these metabolites included butyrate, L-arginine, CO_2, ammonium, ornithine, ethanol, L-glutamine, and glycine. Of these, the latter 3 were also seen in those who achieved remission and the other exchanges were specific to patients who did not achieve remission. Some of these metabolite exchanges, such as with butyrate, resolved posttreatment and most other exchanges persisted. Fecal metabolites also differed between responders and nonresponders, with higher levels of 3-indole propionic acid, L-tyrosine, and 3-hydroxyphenylacetic acid at baseline among those who achieved remission and higher levels of pyric acid in the no-remission group.

One prior study examined the role of microbial composition in predicting response to ustekinumab therapy. Doherty et al[27] performed 16s rRNA sequencing from fecal samples of patients treated in a phase 2 clinical trial (CERTIFI) comparing ustekinumab induction and maintenance to placebo. Stool samples were obtained at baseline and weeks 4, 6, and 22. A combined random forest predictive model that incorporated clinical and baseline microbial data performed significantly better than clinical data containing models alone (AUC = 0.84 vs 0.62). In fact, the addition of clinical data did not add predictive value to microbiome-only predictive models (AUC = 0.838). Two OTUs were significantly different between those in remission at week 6 and those with active CD: *Bacteroides* (P = 0.022) and *Faecalibacterium* (P = 0.003). Patients in remission at week 6 also had a higher alpha diversity at baseline than did those with active disease. During maintenance therapy through week 22, microbiome-based models could also classify disease activity states based on an abundance of *Faecalibacterium*, *Blautia*, *Clostridium XIVa*, *Ruminococcaceae*, and *Roseburia.*

Disease Progression

Two pediatric inception cohorts with serial sampling of the microbiome in mucosa and stool provided insights into the potential predictive value of microbial markers in identifying disease progression.[14, 23] A total of 913 children were enrolled at diagnosis of CD, most of whom had a nonstricturing, nonpenetrating phenotype.[14] At follow-up, 54 and 24 patients had stricturing and penetrating complications respectively, and 21% received anti-TNF therapy. Interestingly, both *CBir1* positivity and the presence of anti-saccharomyces antibodies was associated with a risk of stricturing or penetrating disease behavior. Expression of genes regulating acute inflammatory response to microbes was induced at baseline in those who developed penetrating disease. Specifically, fecal abundance of *Rothia* and *Ruminococcus* was associated with the development of stricturing complications and *Collinsella* abundance was greater at baseline in patients who developed penetrating disease. The PROTECT cohort recruited 400 children

TABLE 2. Challenges and Opportunities in Studies of Microbiome-Based Biomarkers in IBD

Challenge	Proposed Solutions
Heterogeneity in participants	Standard definitions for inclusion criteria
Variability in outcomes	Use validated outcomes, preferably relying on objective markers
Small cohorts	Multicenter and international collaborations
Competing external influences (diet, antibiotics)	Develop a minimum standard of suggested covariates that need to be ascertained and controlled for
Lack of validation of findings	Harmonization of protocols to ensure comparability across cohorts and pooling data

with newly diagnosed ulcerative colitis who were initiated on mesalamine (+/– corticosteroid therapy).[23] Of these, 38% achieved corticosteroid-free remission at week 52. Abundance of *Ruminococcaceae* and *Sutterella* at baseline predicted week 52 remission outcomes (odds ratio = 1.43, $P = 0.04$; and odds ratio = 0.81, $P = 0.05$, respectively).

Limitations of Existing Data and Suggestions for Future Research

1. An important limitation of existing data is heterogeneity in the selection of patients including variation in disease activity, severity, and ongoing treatments (Table 2). This limitation has the potential to influence effects beyond the biological parameter being studied, leading to confounding and misinterpretation of data. Thus it is important in future studies to use well-established criteria using accepted definitions to ensure comparability of data across populations.
2. A second important challenge with existing data is the wide variability in the definition of outcomes. Among the studies that have examined treatment response, outcomes have been variably defined at 4 weeks, 8 weeks, 14 weeks, or 54 weeks. Further, studies have varied in the use of symptom-based disease activity scores, calprotectin normalization (using different thresholds), endoscopic remission, or global physical impression to define the outcome. The varying accuracy of each of these parameters confounds the interpretation of findings and highlights the lack of replicability of results across cohorts. Thus, it is important to formulate accepted definitions for each of the clinically relevant endpoints in the field to facilitate robust validation of identified signals.
3. Studies have also varied in the depth of microbial sequencing, from polymerase chain reaction–based methods to 16s rRNA profiling to metagenomics sequencing and strain-level analysis. While decreasing the cost of the more comprehensive sequencing methods that also define bacterial function and the recognition of variation in biologic effect even at the level of individual strains, the development of microbiome-based therapies requires more comprehensive microbial profiling to truly identify modifiable targets. In addition, most studies have relied on the comparison of relative microbial abundances, which, in addition to the species of interest, may be influenced by total microbial counts and changes in the abundance of other species. Newer methods such as quantitative microbial profiling may be important to identify additional changes pertinent to the development of disease.
4. The published studies have differed on the amount of information collected regarding relevant important confounders including antibiotic use, inflammation severity, diet, and medications (to name a few). This inconsistency complicates an understanding of the impact of the biologic parameter being studied and of such external influences. To reduce this concern, there must be transparent reporting of this information in every study and an establishment of minimum acceptable standards.
5. The lack of reproducibility of associations is an important limitation of existing studies of the microbiome in IBD. Most prediction studies have failed to include independent external validation cohorts, in part limited by the costs of such cohort accrual and profiling, and by the heterogeneity in study procedures between different cohorts. For example, although genetic association studies in IBD could make rapid strides in identifying potentially causal gene variants by the relative ease and consistency of defining a disease state (CD, UC, or control), microbiome-based prediction studies have required more nuanced, time-varying definitions of outcomes (such as treatment response) that have differed between cohorts, limiting the ability to pool data.
6. Existing tools for profiling the taxonomic or functional profile of the microbiome or the metabolome are not readily translatable to point-of-care tests in the clinic because of their logistical challenges in profiling single samples cost-effectively and the high volume of data generated with each profile. Further research is essential to move predictive associations identified in the research arena to clinical care. The emergence of tools such as paper-based technology for microbiome profiling may prove to be cost-effective and applicable to direct patient care.[45]

CONCLUSIONS

In summary, given the likely central role of the microbiome in the pathogenesis of IBD, it is intuitive that microbiome-based biomarkers may be particularly relevant in stratifying patients with these complex diseases. Indeed, intriguing preliminary data have supported the role of such biomarkers in predicting many important clinically relevant outcomes. However, existing studies are limited by small sample sizes and significant heterogeneity preventing robust validation. Similar to what has been achieved and is highly critical for advancing science in other fields (such as genetics), multicenter collaborations of relatively homogeneous populations and uniform accepted definitions controlling for confounders are essential to move such microbiome-based biomarkers from a research endeavor to clinical practice.

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