Dysbiosis in Functional Bowel Disorders

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Keywords

Functional gastrointestinal diseases · Irritable bowel syndrome · Microbiota · Dysbiosis · Probiotics · Fecal microbiota transplantation

Abstract

Functional bowel disorders (FBD) resemble a group of diseases of the gastrointestinal (GI) tract that are without a clear pathogenesis; the best known is probably the “irritable bowel syndrome” (IBS). Only recently we have been able to explore the role of the gut microbiota in FBD due to progress in microbiological analytic techniques. There are different ways to explore the role of the gut microbiota and its dysbiosis in FBD. Comparison of the microbial composition in a group of patients with FBD, for example, with IBS to a group of healthy volunteers is one way. Studies have shown that the microbiota in FBD is different from that of healthy controls, but the recorded differences are not necessarily specific for FBD, they may also occur in other diseases. Another approach to explore the role of the gut microbiota in FBD is to challenge the existing “flora” with novel bacteria (probiotics) or with nutritional substrates that stimulate bacterial

growth (prebiotics). More than 60 such trials including several thousand patients have been performed in IBS. These studies have produced mixed outcome: some probiotics appear to be better than others, and some appear to work only for a part of the IBS symptoms and not for all. An extreme form of this approach is the transfer of an entire microbiota from 1 healthy person to another, called fecal microbiota transplantation. This has rarely been tested in FBD but is not without risk in benign disorders.

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Introduction

Functional bowel disorders (FBD) resemble a group of diseases of the gastrointestinal (GI) tract (esophagus to anus – swallowing problems to defecation disorders) without a clear pathogenesis, which are quite frequent and bothersome for the individual; the best known condition is probably the “irritable bowel syndrome” (IBS). Be-

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cause many FBD symptoms are associated with eating or elimination of certain foods, it has been assumed from very early times that food eaten on the one hand and bacteria involved in its processing on the other hand may play a key role in FBD origin and/or its clinical picture. But only recently we have been able to explore the role of the gut microbiota in FBD due to progress in microbiological analytic techniques.

The term “dysbiosis” implies that the microbiota – the community of microorganisms living in an environment, for example, in the human GI tract – is unbalanced, that the composition of the different bacterial genera living there regularly (called commensal bacteria, to distinguish them from pathogen bacteria) is disturbed to the extent that some or many bacteria are missing and others may become more prevalent or dominant. While we have learned of many new bacterial species and their role in health and disease, unfortunately the proper “balance” of the several hundred different bacterial species (and the total of trillions of bacteria) is unknown, as is the role of single bacterial species and their metabolic functions for a healthy gut.

There are different ways to explore the role of the gut microbiota, and its balance or unbalance (dysbiosis) is FBD. One method can be to compare the microbial composition in a group of patients with FBD for example, with IBS to a group of healthy volunteers, but in such a study, extreme care has to be taken that the “controls” are not only of similar age and gender, but also of the same cultural background and nutritional habits, since these factors affect the microbial composition and diversity.

Another approach to explore the role of the gut microbiota in FBD is to challenge the existing “flora” with novel bacteria (probiotics), or with nutritional substrates that stimulate bacterial growth (called prebiotics), especially in randomized clinical trials (RCT) where only a fraction of patients receive the true challenger, while the others receive a placebo. This has been done excessively in FBD, especially in IBS, with more than 60 trials including several thousand patients. These studies have produced a mixed outcome: some probiotics appear to be better than others and some appear to work only for a part of the IBS symptoms and not for all.

Another approach – not often performed in RCT – is the transfer of the entire microbiota from one healthy person to another and this approach is called fecal microbiota transplantation (FMT). This has rarely been tested in FBD but is not without risk in benign disorders.

We will review these 3 approaches with respect to their outcome in FBD, with a specific emphasis on the IBS, which is the most relevant of these disorders [1]. A central role of the microbiota in the other important FBD, functional dyspepsia, has not been proposed so far [2].

Microbiota Assessment in IBS

IBS clinically manifests with abdominal pain associated with other GI symptoms like constipation, diarrhea, bloating, and flatulence in the absence of an organic cause of symptoms; it is one of the most frequent disorders in the general population and in private practice, as seen by primary care physicians as well as gastroenterologist [3]. Across 42 countries worldwide, the pooled prevalence is 11.2% [1, 4]. The pathophysiology of IBS is believed to be consequence of the interplay between gut-brain axis disturbances, local immune changes and inflammation in the gut wall, and changes in gut permeability and gut microorganisms’ composition. The gut microbiota is regarded as a key player in the pathophysiology, and probiotics is seen as a putative agent that possibly could affect several targets in this assembly, and thus may induce better and longer remissions in the chronic and recurrent condition. Even with more hypothetically driven mechanisms of action of probiotics onto brain functions and conditions [5], they may improve central functions through stress-moderating effects [6].

While the search for biomarkers of the IBS has – from the very beginning [7] – also included the interior milieu (microbes, viruses, fungi) of the GI compartments, only the surge of molecular technologies in the last decade has brought about significant progress in our understanding of the role of the microbiota (in a broader sense) and commensal bacterial colonization (in a narrower view) for the hosts’ health and disease [8]. Despite global agreement on its importance however, agreement among researchers is still limited by methodological and technical handicaps and restrictions [9].

Early summaries of previous investigations to characterize the intestinal microbiota in IBS [10] have listed individual bacterial species to be either increased in abundance (such as *Lactobacillus*, *Veillonella*, and *Enterobacteriaceae*), or decreased (*Bifidobacterium*, *Clostridium*) in comparison to that of healthy controls, or of patients with other, for example, somatic disorders such as inflammatory bowel diseases (IBD) [11]. However, if the investigations were based on culturing techniques, then they are regarded as less reliable.

Fig. 1. Phylogenetic tree representing the diversity of the human intestinal microbiota. The typical intestinal taxa are shown and presented in bold when found to be significantly different between IBS patients and healthy subjects and the direction of the change

is indicated by the arrows (down arrow, higher in healthy; up arrow, higher in IBS). The reference bar indicates 10% sequence divergence (reproduced with permission from [13]).

A more thorough investigation based on deep sequencing of the microbiome (the genes regulating bacterial functions, 150-fold in numbers as compared to the human genome [12]) has substantiated the view that the IBS microbiota is deviant in the number and diversity of bacterial families found in the gut [13] (Fig. 1). The initial sample of $n = 100$ IBS patients and controls has meanwhile been updated [8] and has confirmed these findings. This has led to believe that a measure of the *Bacteroidetes-Firmicutes* ratio may be a reliable indicator of dysbiosis in IBS [14].

Evidently, the most effective way of manipulating the microbiota is by bringing about a change of diet [15, 16], and therefore, in studies not controlling for diet, the microbiotic “dysbiosis” may reflect variability in diet or disease-specific dietary habits that are quite common in IBS [17] rather than a disease marker – a “hen or egg” phenomenon common also to other putative biomarkers [18]. Controlling for diet is however, a rather tedious task

not only in IBS, as both regular dietary habits (e.g., a vegetarian/vegan versus an omnivore diet [19]) as well as short-term change of food intake [20] contribute to the microbiota composition, for example, during travel [21, 22]. At best, nutritional trials, for example, with pre- or probiotics (see below) can request patients to maintain their regular diet during the intervention to average out such variability of dietary habits, but this is difficult to control. Single time-point assessments of the microbiota compositions thus offer little insight into the disease mechanisms on the one hand and the microbiota contribution to symptom development on the other, neither in IBS nor in other diseases.

What also needs to be addressed is whether “dysbiosis” in IBS or any other of the reported changes are specific to IBS, or whether similar changes of microbiota diversity can be found in more diseases and conditions; in the latter case, this would highlight their dependence on other conditions (e.g., diet) and would question their

relevance as disease biomarkers. The claimed distorted *Bacteroidetes/Firmicutes* ratio (in favor of *Firmicutes*) in IBS was first identified in obesity [23] and had been shown to normalize with weight loss in mice [24] and men [25]; in obesity, this finding was not confirmed in a recent meta-analysis [26]. A dysbalance of the ratio was also reported in a study of depressed patients from China [27] but was not replicated in another study from the same country [28]; in fact, the latter found nearly the opposite. This already casts doubts about its relevance for FBD.

Another limiting factor of the assessment of the composition and diversity of the microbiota, especially in FBD of IBS-type, is the fact that it assumes stability across time within individuals (even with stability of nutritional habits), but this has been questioned recently: Assessment of the microbiome at different bodily niches (ear, nose, teeth, tongue, throat, gut, genitals) at up to 4 time points (weeks to months apart) in 265 individuals revealed the stabile nature of some bacteria (e.g., *Bacteroidetes*), but instability of other bacterial families (e.g., *Firmicutes*), especially for the stool microbiota, so that the frequently claimed clinical relevance of the *Bacteroidetes/Firmicutes* ratio when assessing “dysbiosis” may be dubious at least, if not useless [29]. The authors conclude that individuals may “... carry a ‘personal equilibrium’ among *Bacteroidetes*, with a group of phylogenetically diverse, temporally variable *Firmicutes* fluctuating atop if this core” (ibid., p 5).

Finally, assessments of gut dysbiosis via a “dysbiosis index” as is practice in some medical and laboratory routine, for example, the dysbiosis index developed by GA, Oslo, Norway [11] are based on similar assumptions: that assessing a variety of different bacteria (in this case: targeting >300 bacteria on different taxonomic levels) may result in a microbiota profile that can be used to identify its sensitivity in comparison to healthy control subjects but also its specificity in comparison to other diseases. While it has been shown that the dysbiosis index can predict response to a food challenge (low FODMAP diet) but not to conventional dietary intervention in IBS [30], the predictive value was low, and specificity has not been tested yet.

Probiotic Therapy in IBS

Living microorganisms from dairy products were used for centuries to modify bowel functions, to treat bowel disorders, or to improve the health state in general, but they received their current name “probiotics”

(“supporting life”) only in the 1950s. The modern definition by the International Scientific Association for Probiotics and Prebiotics describes probiotics as living organisms that, when ingested in sufficient amounts, may be beneficial for the host [31]; a similar und updated definition of prebiotics has been published recently [32]. It avoids any speculations regarding the possible mechanisms of action, as the latter changed substantially during the last decades with the development of modern research techniques (molecular analysis instead of culturing technology) and with growing knowledge of the interactions between human body and these microorganisms [33].

The interest in probiotic studies and probiotic treatment rapidly increased during last 20 years from only 9 papers in 1995 to more than 1,500 papers in 2014 that can be found in the PubMed library with the search term “probiotics”. One of the reasons for that was a growing interest in the so-called functional GI disorders, such as IBS, functional dyspepsia, functional constipation, and so on [34] during the same time span. These disorders have a high impact on the quality of life of affected patients, are associated with enormous economic burden for society, and usually do not respond well to conventional treatment strategies, including novel and traditional medications, laxatives, bulking agents, and dietary interventions [35]. Probiotics appear to offer an alternative with a high safety margin, but they may cost as much as drugs or more. Among the disorders that have been shown to benefit from probiotic treatment in placebo-controlled, double-blinded, and randomized studies are antibiotic-associated diarrhea [36], traveler’s diarrhea [37], rota-virus-induced diarrhea [38], and *Clostridium difficile*-associated diarrhea [39]. Beneficial effects have also been shown in diarrhea in children [40]. Some bacterial strains such as *Escherichia coli* Nissle showed efficacy in chronic constipation [41] or in the maintenance of remission in ulcerative colitis [42].

Our recent systematic review [43] of 56 randomized and placebo-controlled trials in IBS reported between 1989 and 2015 and that have been included in (or excluded from) 9 meta-analyses published between 2008 and 2015 left us with questionable evidence about their efficacy, and raised questions with respect to the quality of both the RCTs as well as meta-analyses. In this study, we summarize the review and raise critical questions of relevance for doctors facing the difficulty to treat IBS patients (for full references of all papers and meta-analyses see [43]). Because of the strict definition of probiotics as living bacteria, studies using inactivated bacteria and cell

fragments were excluded from this review, despite evidence for their clinical efficacy [44, 45].

We performed a systematic review rather than a meta-analysis that also follows predefined criteria for paper selection and review but do not use statistical methods for comparing their efficacy. This approach allowed us to include not only all RCTs that have been included in previous meta-analyses but also those that were not suitable for statistical analyses but could provide important information on the efficacy of probiotics in patients with IBS.

Multi-Strain Preparations

One half of the selected papers present the results of trials with probiotic preparations that consist of multiple strains that often also belong to the different species. The idea behind multi-strain or “multi-species” preparation is that probiotic mixtures may provide a better chance of survival of exogenous bacteria in the GI tract but also that different microorganism may develop a synergic action that will enhance the beneficial effect of the whole compound for the host. As the general discussion about this concept continues with some studies providing evidence in favor of multi-species probiotics [46], to our knowledge, there are no placebo-controlled head-to-head trials of single- vs. multi-strain preparations in IBS.

There is large diversity between trials: Most of the multi-strain preparations were nutritional supplements from different suppliers worldwide. The same products may be available in different countries under different brand names, with different labels and recommended for different indications, although approval of indications is not common in nutritional supplements. While some strains (such as *Lactobacillus acidophilus*) are included in almost all preparations, many if not most of the strains are used exclusively in a few combinations (and are probably protected by patents). The use of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* as starting cultures for yoghurt production is noted in some papers, but in the other cases, it remains unclear whether these strains were included: they were not regarded as probiotics until 2014, when the International Scientific Association for Probiotics and Prebiotics consented that they are probiotics by definition [31].

Other aspects of these studies also varied substantially: the number of participants ranged from 24 to 186, and treatment duration from 7 days to 6 months. As a common reporting period for endpoint assessment has not been agreed upon (e.g., after 4 or 8 weeks of intervention), it is difficult to compare studies with each other regarding

their outcomes. Not all studies reported the dosage of the microorganisms in the preparations and based on the available information, the difference between the lowest and the highest dose was almost thousand fold.

In summary, 14 of the 27 studies that used multi-strain preparations reported negative outcomes on global symptoms; however, some symptoms (bloating and satiety) were occasionally reported as being responsive to treatment. Quality of life improved according to a study, but symptomatic improvement was not different from placebo. Thirteen studies demonstrated positive effect on global symptom reports. While the size of the study populations has increased over the years, this did not affect the (positive or negative) outcome of the studies, as more patients were included in the studies reporting negative outcomes than in the studies with positive outcomes. The balance of positive and negative studies indicates a rather arbitrary and random result rather than an overall effective outcome of treatment attempts using multi-strain probiotics in IBS.

Single-Strain Preparations

Single-strain probiotics seem to be more suitable to demonstrate efficacy, as they come closer to traditional drug studies. We found 29 papers published between 1989 and 2014 that applied single-strain probiotics to modify IBS symptoms (Fig. 2).

All studies demonstrate large variability with respect to various design features: Although most studies used a parallel group design, 4 of them were conducted using cross-over design in which patients are included both in treatment and control arm consecutively. This is frequently used for motivational purposes, since patients are easier to recruit when they are offered effective treatment, at least for 1 period of the trial. However, the assumption that the data in both periods are equal and can simply be compared may be biased by un-blinding and conditioning effects [47].

Studies are also different regarding the treatment duration ranging from 4 to 12 weeks and the number of participants: the lowest number of included patients was $n = 12$ and the largest $n = 362$. A low number of participants means that there is low statistical power of conclusions and thus low reliability of data. Moreover, the amount of living organisms in the preparations varied by almost the factor of 1,000, ranging from 1×10^8 [48] to 8×10^{11} [49].

But the most impressive differences could be seen when the different species used to modify IBS symptoms were analysed separately: 6 studies used *Lactobacillus*

Fig. 2. Distribution of single- and multi-strain probiotic preparation RCTs with positive and negative global outcome in IBS. Numbers refer to the numbering in the reference list of the original paper [43]. IBS, irritable bowel syndrome; RCT, randomized clinical trial.

plantagus [50–55], however, of different origin and subspecies. This is of particular importance, since recent studies suggest that probiotic effects are strain-specific and the strains belonging to the same species may have no or different probiotic effect [56]. Other lactobacillae strains are used only in single trials, like *Lactobacillus brevis* [57], *Lactobacillus acidophilus* [58, 59], *Lactobacillus reuteri* [60], *Lactobacillus rhamnosus* GG [61], and *Lactobacillus casei* (CLR35) [62].

A similar picture emerges with bifidobacteria: Individual trials have used *Bifidobacterium bifidum* (MIMBb75) [63], *Bifidobacterium lactis* [64], *Bifidobacterium animalis* [65], and *Bifidobacterium infantis* [48, 66]. Of the 4 studies studying the effect of yeast *Saccharomyces* on IBS, 3 used subspecies *boulardi* [49, 67, 68] and one *cerevisiae* [69], but as in a case with lactic acid bacteria it is not possible to recognize if they belong to the same strain and thus possess similar qualities. Only a single study used *Streptococcus faecium* strain [70] and 2 used different *Escherichia coli* strains, *E. coli* Nissle [71], and *E. coli* [72].

When all studies are viewed in balance, it reveals that 16 of the 29 studies yielded a negative or at least partly negative outcome (Fig. 2). Taking all patients in positive and negative studies into account, more patients had a benefit from probiotics than those who did not. One direct comparison of a *lactobacillus* versus a *bifidobacteri-*

um strain, and both against placebo [73] revealed the *bifidobacterium* to be effective, while the *lactobacillus* was ineffective in relieving IBS symptoms.

Overall, studies using lactobacillae strains do not appear to be effective: 9 out of 13 studies reported negative outcome. In contrast, bifidobacteria appear to be more effective in IBS, with 4 out of 6 studies yielding positive results. All 4 studies using *Bacillus coagulans* were positive, although small in number. All others were either negative (*Saccharomyces*) altogether or with mixed results of only 1 or 2 studies that do not allow final conclusions, but require independent confirmation.

This limited evidence for clinical efficacy of probiotics in general – and specific probiotics in some instances – does not imply that probiotics systematically alter the commensal microbiota as their main mechanisms of action. The above listed probiotics studies provide little insight into these mechanisms and leave this question open for further and more thorough investigations in the future. While it has been shown in some cases that the oral intake of probiotics may allow the living bacteria to survive the GI passage and be identified in stool samples [74], their presence is usually diminished shortly after their intake has been terminated, and a consistent manipulation of the commensal microbiota has not been described – therefore, their mechanism of action must

use a different pathway, for example, via immune-stimulation, metabolic effects, or interaction with the enteric nervous system [75]; their discussion is beyond the scope of this review.

FMT in FBD

A further approach to assess (and modulate) the microbiota in IBS has come into focus after it was shown that FMT is very effective in the management of patients with refractory *Clostridium diff.* infections; this has been confirmed in many studies and a number of meta-analyses [76]. For details of the technical aspects of FMT, the reader is referred to respective papers [77, 78]; meanwhile, ethical standards have been discussed [79] and procedural standards have been set by respective organizations, for example, for European gastroenterologists [80], in Canada [81] and in Australia [82].

Almost immediately after the publication of these new concepts, discussion started around the question whether other diseases could profit from FMT as well, and certainly GI disorders such as FBD/IBS and IBD came into focus [83, 84]. Meanwhile and with increasing numbers of respective randomized and controlled trials, the smoke has been lifted and a differential picture emerges, at least for IBD: FMT transplantation appears to be effective in ulcerative colitis, especially with induction [85] or maintenance of remission [86], while in Crohn's Disease, less effect was found [87, 88]. Another potential application is pouchitis [89, 90]. During these studies, a number of mediators of treatment success were identified, among them the donor, the route of administration of the stool transplant, and others [91, 92].

It has, however, been questioned from the very beginning whether benign diseases such as IBS that pose no life-threatening risk onto patients should be subjected to FMT, given that the procedure itself may bear risks: case reports of adverse events [93–96] and the transmission of so far silent enterotoxigenic agents (viruses, bacteria) to the patients [97] are just some of the potential threats [98] as long as the “ideal donor” has not yet been characterized [99]. It has been proposed [100] that in the future, synthetic (engineered) stools composed of known and well-characterized bacterial species serving specific functions [101, 102] could be used, rather than using a mixture of stools of healthy persons as frequent current practice [103].

It has taken quite a while for case reports and uncontrolled observations [83, 104] to be followed by the first

randomized controlled trial of FMT that was recently published [105]. In this study, 60 patients with moderate to severe IBS (>175 points on the IBS Symptom Severity Scale [106]) received feces from 2 donors (mixed); half of them received a fresh preparation while the other half received a mixture of previously frozen samples, while patients on placebo treatment received their own stool. The study was double blinded and evaluated after 3, 6, and 12 months, with improvement in IBS-Symptom Severity Scale of more than 75 points being the primary endpoint. The endpoint was reached in 65 and 43% in the active and placebo group respectively, making this result significant ($p = 0.049$) for the 3-months follow-up but not thereafter.

Summary and Conclusions

The term “dysbiosis” is (still) lacking a consented definition and thus implies only some form of dysbalance of the bacterial composition (or abundance or diversity) when a patient group is compared to healthy control subjects. This dysbalance may or may not be specific for this disease; whether it is representing a disease biomarker or occurs secondary to disease-related behaviors (e.g., nutrition) cannot be judged from single time points of investigation but requires consecutive sampling with simultaneous variation of the disease. The latter can be induced by nutritional interventions, for example, with pre- and probiotics but this does not imply that altering the commensal microbiota is the only way by which probiotics act on gut health: Probiotics have been shown to affect immune functions, metabolic functions, and may as well directly interact with the enteric nervous system at the gut barrier [75]. The marginal action of (only some of the) probiotics on GI functions not only casts doubts on their overall clinical value, but also calls to our attention that perhaps the reason for this lack of efficacy is based on the poor definition of the underlying origin of symptoms in IBS: Similar symptoms (diarrhea-predominant, constipation-predominant, and mixed stool habits with abdominal pain) may be of an entirely different etiology, a fact that is also reflected in the long list of putative biomarkers found to be associated with IBS symptomatology [1], and in such case, a specific probiotic may only be effective in a subset of patients. Except for 1 study [71], none of the listed papers have selected patients by a putative pathomechanism, and hence one cannot expect overall global efficacy beyond chance. Finally, exchanging the

microbiota and replacing it by one from an apparently healthy person may be a final option in therapy refractory and life-threatening diseases, but in our understanding, it cannot be the therapy of choice in FBD, as long as the above-mentioned question of the etiology of symptoms has not been clarified.

This position with respect to dysbiosis of the gut microbiota in FBD may be illustrated by the *Anna-Karenina-Principle*, referring to the first sentence in Lew Tolstói's novel *Anna Karenina*, "*happy families are all alike; every unhappy family is unhappy in its own way*". This has recently been applied also to microbiological research [107], but with respect to FBD it implies, that a complex function (such as digestion) is in balance (called health) only when all its subunits are functioning properly, while unbalance of only one (of the many) may re-

sult in the progression of disease: there is only one way to health, but many ways to develop a disease, especially in FBD.

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