BIOTASCOPE

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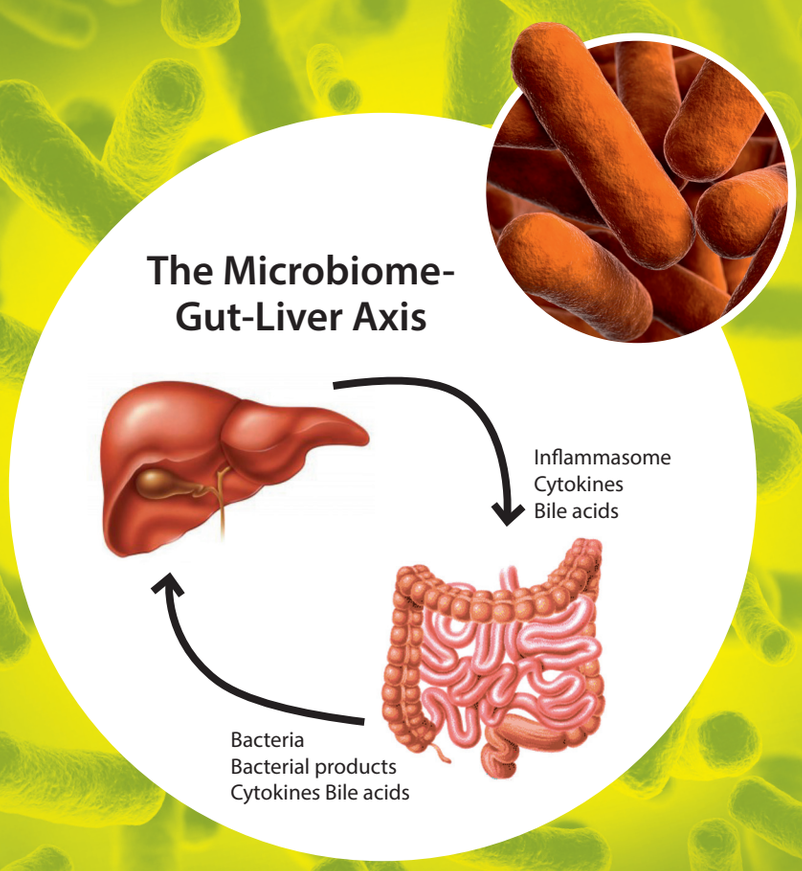
Translational Science in Microbiota



Springer Healthcare

Communications

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Editorial

Dear Colleagues,

We are delighted to introduce the 3rd issue of Biotascope, which covers the complex relationship between the gut microbiome and the host. The fact that the number of microbes inside the human body is 10 times higher than the number of our own cells is simply fascinating. It is therefore reasonable to assume that all of these microbes must have a significant impact on numerous functions and diseases of the human being. With the participation of experts in this area of research, our aim is to discuss various aspects of this interaction in each Biotascope publication.

In this issue, Dr Eamonn Quigley from Houston, Texas, USA, will address the role of the gut microbiome in relation to non-alcoholic fatty liver disease, with a particular emphasis on pathogenetic mechanisms. This is an exciting topic as non-alcoholic fatty liver disease is a common disorder and is closely related to the increasing incidence of obesity.

Many review articles and meta-analyses have been published regarding the involvement of the microbiota in gastrointestinal disorders, including irritable bowel syndrome. In an article entitled "Probiotic Therapy in Irritable Bowel Syndrome", Mazurak and Enck make a particularly relevant contribution to this issue of Biotascope. This manuscript can be defined as the meta-analysis of meta-analyses as it describes the evaluation of nine meta-analyses of randomized controlled trials on the efficacy of probiotic therapy in patients with irritable bowel syndrome during the past 15 years. We also intend to focus on both adult and pediatric topics in each Biotascope and in this issue Ener Cagri Dinleyici from Turkey explores the role of probiotics and synbiotics in treating children with acute infectious diarrhea and what clinical guidelines recommend.

As you are already aware, Biotascope includes summaries of original research articles from renowned scientific journals. Dr Tarkan Karakan reports on relevant topics from the latest literature including how *Faecalibacterium prausnitzii* induces interleukin-10 in dendritic cells and modulates T-cell responses, the respective effects of oral and intravenous iron replacement therapies have on the gut microbiota in patients with inflammatory bowel disease, the correlation between necrotizing enterocolitis and gut microbiota, and, lastly, the role of the host in shaping the gut microbiota via fecal microRNA.

Another important section of the journal reports on scientific concepts discussed during key international meetings. This year, Peru hosted the 20th Congress LASPGHAN in Lima and our friend from Peru, Dr Aldo Maruy – a member of the International Study Group of Probiotics (ISGoP), wrote a summary of the meeting for our pediatric colleagues. Furthermore, in line with the first Biotascope issue, Dr Claudia Herrera de Guise and Dr Francisco Guarner wrote a comprehensive review on the United Gastroenterology Week 2015. This year, a total of 55 abstracts focused on gut microbiota and 14 research papers dealt with the gastrointestinal effects of probiotic supplementation. Subjects covered in this article include fecal microbiota transplantation, inflammatory bowel disease, celiac disease, and chronic pancreatitis.

We welcome any feedback and are keen to hear about your views on the themes discussed as well as topics you would like to see included in future issues of Biotascope; you can contact us at journal@biotascope.com.

Best wishes, from the International Study Group of Probiotics (ISGoP).

Sincerely,

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NON-ALCOHOLIC FATTY LIVER DISEASE; THE ROLE OF THE GUT MICROBIOME

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INTRODUCTION

The liver is strategically placed to encounter those microbes or microbial components that may have traversed (translocated across) the gut barrier and entered the portal circulation. Consequently, changes in the gut microbiome and/or intestinal barrier integrity coupled with portal-systemic shunting and impaired liver function, as seen in liver disease, will lead to major complications such as portal-systemic encephalopathy, spontaneous bacterial peritonitis, and systemic sepsis. Indeed, changes in the small intestinal microbiome in the form of small intestinal bacterial overgrowth (SIBO) have been recognized in chronic liver disease for decades. In addition, the role of bacterial metabolism of dietary protein in the pathogenesis of portal-systemic encephalopathy was conclusively demonstrated over 50 years ago [1]. When reviewing the literature from a search for various liver diseases in which the microbiome is implicated, an interplay between three factors emerges as a recurrent theme: an altered microbiome (dysbiosis), impaired intestinal barrier function, and an altered or amplified host immune response. This is not to say that other factors (e.g. environmental, genetic) are not relevant, but that the aforementioned factors seem pivotal to the proposed role of the microbiome in diseases as diverse as alcoholic liver disease, intestinal failure-associated liver disease, and primary sclerosing cholangitis [1-3].

The exploration of the microbiome and its various biological activities has of course been facilitated by rapidly evolving technologies, which permit, firstly, the rapid identification of the composition of the microbiome and, secondly, the definition of its functional capacity and metabolic profile [4].

THE GUT MICROBIOME AND NON-ALCOHOLIC FATTY LIVER DISEASE

An exploration of the changes in the composition and function of the microbiome in non-alcoholic fatty liver disease (NAFLD; Figure 1) became inevitable once a role for gastrointestinal bacteria in obesity and metabolic syndrome, disorders that so frequently coexist with NAFLD, was established. Whilst examining the role of the microbiome in these disorders, the critical role of diet in modifying the composition and metabolism of the microbiome must be considered, both in the short-term and in the long-term [5-9]. The microbiota can also modulate bile acid metabolism and, consequently, the de novo synthesis of bile acids in the liver through feed-back control. Given the recognition of the importance of bile acids as signaling molecules [10], microbiome-mediated effects on bile acids could also impact on factors such as insulin sensitivity and hepatic fat metabolism [11], each highly relevant to the pathogenesis of NAFLD.

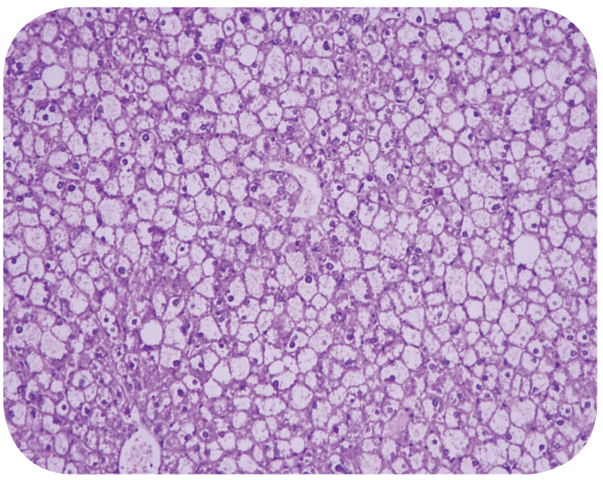

Figure 1: Histological features of steatosis in non-alcoholic fatty liver disease. *Hematoxylin and eosin staining. Note the diffuse steatosis throughout the liver.*

Support for the relevance of the microbiome in the pathogenesis of NAFLD also comes from the documentation of its role in two disorders which share considerable pathologic similarity to NAFLD: alcoholic liver disease [12] and total parenteral nutrition (TPN)/intestinal failure-associated liver disease [13].

Alterations of The Gut Microbiome in Non-Alcoholic Fatty Liver Disease/Non-Alcoholic Steatohepatitis

SIBO has been recognized in liver disease for decades and implicated in the development of various complications of cirrhosis through bacterial translocation across an impaired gut barrier [14,15]. In experimental models of NAFLD, SIBO has been shown to promote both steatosis and inflammation [16, 17] and, in clinical studies, SIBO has been linked to non-alcoholic steatohepatitis (NASH) [18, 19].
The findings that toll-like receptor 4 (TLR-4) is upregulated in NASH [19] and that circulating levels of tumor necrosis factor-alpha (TNF-α) are increased provide credence to the hypothesis that Gram-negative bacteria in the gut lumen are promoting an inflammatory response in NAFLD [18].

But what of the colonic microbiome in NAFLD/NASH? This information has been largely derived from fecal sampling, an approach that, while convenient, may not represent interactions at the microbiome-epithelium interface [20].
Furthermore, one must also be cautious in the interpretation of microbiome changes among subjects with advanced NASH; such alterations may simply reflect the consequences of advanced liver disease, *per se* [21, 22].

In NAFLD, Raman and colleagues described an over-representation of Lactobacillus species and selected members of the phylum Firmicutes (Lachnospiraceae; genera, Dorea, Robinsoniella, and Roseburia) and an under-representation of one member of the phylum Firmicutes (Ruminococcaceae; genus, Oscillibacter) [23]. In another study, Mouzaki and colleagues noted an inverse relationship (which was independent of diet and body mass index) between NASH and percentage of Bacteroidetes in the stool; an observation consistent with the hypothesis that Firmicutes, the other dominant phylum, are more efficient in calorie extraction [24]. However, these observed changes may be non-specific, and merely reflect the impact of obesity or the metabolic syndrome on the composition of the microbiome. It appears that some microbial signatures are specific for NAFLD/NASH; Zhu and colleagues found that the populations of Proteobacteria, Enterobacteriaceae, and Escherichia differed significantly between obese and NASH subjects [25]. Interestingly, these authors also noted significantly elevated blood ethanol levels among NASH patients alone, suggesting that the microbiome was a source of endogenous alcohol production [25].

Mechanisms of Microbiome-Mediated Effects in NAFLD/NASH

It seems clear, that SIBO and a disturbed fecal microbiome are common in NAFLD/NASH [23-27]. The question is: How does the microbiome influence the development of steatosis, in the first instance, and thereafter the progression to NASH? Table 1 lists a number of factors that might contribute to the role of the microbiome in NAFLD/NASH.

Table 1

Factors contributing to the role of the microbiome in NAFLD/NASH

- **Altered gut epithelial permeability**
- **Choline metabolism**
- **Endogenous alcohol production**
- **Release of pro-inflammatory cytokines**
- **Up-regulation of hepatic Toll-like receptors**
- **Alterations in bile acid metabolism**

Though modifications in gut permeability have been reported in a number of studies and linked to the release of pro-inflammatory cytokines [27], the influence that these changes in barrier function may have on the pathogenesis of NAFLD or progression to NASH remains to be defined. Alterations in bacterial metabolism of choline have been linked to the development of steatosis [28] and the bacterial production of ethanol has also been implicated in NAFLD [25, 29]. Bacterial activation of pro-inflammatory cytokines, such as TNF-α, via TLR, may play a critical role in the progression of steatosis to NASH [30]. Further studies in experimental animal models and humans have confirmed the critical role of hepatic TLR up-regulation in the pathogenesis of NAFLD/NASH [31-34]. The effects of the microbiome on bile acid turnover and metabolism could also be relevant to the development and/or progression of NAFLD/NASH. In an animal model, manipulation of the microbiome was shown to result in an increase in conjugated bile acid metabolites that inhibited intestinal farnesoid X receptor (FXR) signaling and led to a reduction in the accumulation of hepatic triglyceride [35].

It is critical to understand that the concept of a microbiome-gut-liver axis (Figure 2) is bidirectional; just as intestinal factors can influence liver structure and function, a variety of hepatic factors; including the inflammasome, cytokines generated in the liver and changes in bile salt synthesis, can also influence the gut and intestinal microbiome function [2, 3].

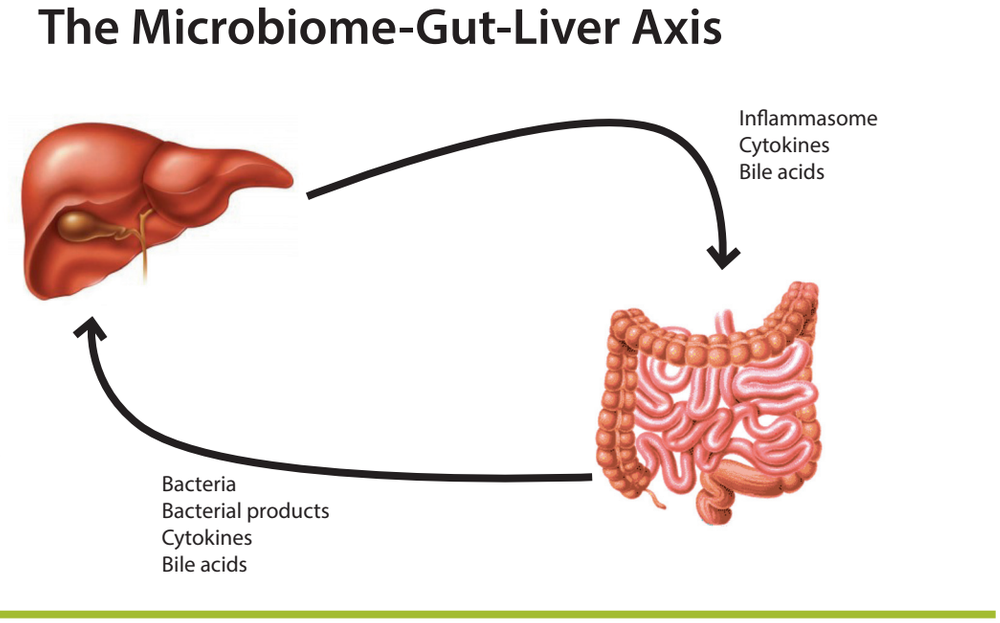

Figure 2:
The microbiome-gut-liver axis.

This axis should be viewed as bidirectional with intestinal factors influencing liver structure and function and hepatic factors impacting on gut function and microbiome composition and metabolism.

SUMMARY

Three factors seem critical to the role of the microbiome in NAFLD/NASH:

1. The microbiome and its metabolic products (e.g. ethanol, deconjugated bile acids, products of choline metabolism). Both SIBO and an altered colonic microbiome may be relevant but their relative contributions remain to be defined.

2. The intestinal barrier.

3. The immune response in the gut and in the liver where TLRs and more specifically TLR-4, appear to play a key role by influencing pathways that control the generation of pro-inflammatory cytokines.

Delineating the role of the microbiome and the host response is critical to the development of interventions that might enhance remission or even reverse the development of NAFLD and its progression.

Already, there is a considerable volume of animal data to indicate that modifying the microbiome through the use of probiotics and prebiotics may alter the natural history of NAFLD [17]; however, the evidence base that supports the development of microbiome-modulating therapeutic strategies in humans remains slim and unconvincing [36, 37].

While considerable progress has been made in identifying a role for the microbiome in NAFLD/NASH, there are still many issues to address, such as the nature and location of the altered microbiome (i.e. small intestine, or colon, or both), the specificity of deficits in intestinal integrity to NAFLD/NASH versus liver disease, in general, the metabolic pathways that are key to the influence of the microbiome and, finally, the therapeutic interventions that are likely to be of benefit to patients.

References

(1) Quigley EM, Stanton C, Murphy EF. The gut microbiota and the liver. Pathophysiological and clinical implications. J Hepatol. 2013;58:1020-7.

(2) Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. Gastroenterology. 2014;146:1513-24.

(3) Chassaing B, Etienne-Mesmin L, Gewirtz AT. Microbiota-liver axis in hepatic disease. Hepatology. 2014;59:328-39.

(4) Fraher MH, O'Toole PW, Quigley EMM. Techniques used to characterise the intestinal microbiota: a guide for the clinician. Nat Rev Gastroenterol. 2012;9:312-22.

(5) Hildebrandt MA, Hoffman C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Knight R, et al. High fat diet determines the composition of the murine gut microbiome independently of obesity. Gastroenterology. 2009;137:1716-24.

(6) Clarke SF, Murphy EF, Nilaweera K, Ross RP, Shanahan F, O'Toole PW, et al. The gut microbiota and its relationship to diet and obesity: new insights. Gut Microbes. 2012;3:186-202.

(7) Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, et al. Gut microbiota composition correlates with diet and health in the elderly. Nature. 2012;488:178-84C.

(8) Hacquard S, Garrido-Oter R, González A, Spaepen S, Ackermann G, Lebeis S, et al. Microbiota and host nutrition across plant and animal kingdoms. Cell Host Microbe. 2015;17:603-616.

(9) Doré J, Blottière H. The influence of diet on the gut microbiota and its consequences for health. Curr Opin Biotechnol. 2015;32:195-9.

(10) Sayin SI, Wahlstrom A, Felin J, Jantti S, Marschall M-U, Bamberg K, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metab. 2013;17:225-235.

(11) Li F, Jiang C, Krausz KW, Li Y, Albert I, Hao H, et al. Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. Nat Commun. 2013;4:2384.

(12) Szabo G, Bala S, Petrasek J, Gattu A. Gut-liver axis and sensing microbes. Dig Dis. 2010;28:737-44.

(13) Quigley EMM, Marsh MN, Shaffer JL, Markin RS. Hepatobiliary complications of total parenteral nutrition. Gastroenterology. 1993;104:286-301.

(14) Quigley EMM. Gastrointestinal dysfunction in liver disease - gut-liver interactions revisited. Dig Dis Sci. 1996;41:557-561.

(15) Fukui H. Gut-liver axis in liver cirrhosis: How to manage leaky gut and endotoxemia. World J Hepatol. 2015;7:425-42.

(16) Abu-Shanab A, Quigley EM. The role of the gut microbiota in nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol. 2010;7:691-701.

(17) Quigley EM, Monsour HP. The gut microbiota and nonalcoholic fatty liver disease. Semin Liver Dis. 2015 Aug;35(3):262-9.

(18) Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cumming AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxemia, and tumor necrosis factor alpha in the pathogenesis of nonalcoholic steatohepatitis. Gut. 2001;48:206-211.

(19) Abu Shanab A, Scully P, Crosbie O, Buckley M, O'Mahony L, Shanahan F, et al. Small intestinal bacterial overgrowth in non-alcoholic steato-hepatitis; association with toll-like receptor 4 expression and plasma levels of interleukin 8. Dig Dis Sci. 2011;56:1524-34.

(20) Bajaj JS, Hylemon PB, Ridlon JM, Heuman DM, Daita K, White MB, et al. Colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy and is linked to cognition and inflammation. Am J Physiol Gastrointest Liver Physiol. 2012;303;G675-85.

(21) Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, et al. Alterations of the human gut microbiome in liver cirrhosis. Nature. 2014;513:59-64.

(22) Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. J Hepatol. 2014;60:940-7.

(23) Raman M, Ahmed I, Gillevet PM, Probert CS, Ratcliffe NM, Smith S, et al. Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2013;11:868-75.

(24) Mouzaki M, Comelli EM, Arendt BM, Bonengel J, Fung SK, Fischer SE, et al. Intestinal microbiota in patients with nonalcoholic fatty liver disease. Hepatology. 2013;58:120-7.

(25) Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology. 2013;57:601-9.

(26) Wong VW-S, Tse C-H, Lam TT-Y, Wong GL-H, Chim AM-L, Che WC-W, et al. Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis – a longitudinal study. PLoS ONE. 2013;8:e62885.

(27) Jiang W, Wu N, Wang X, Chi Y, Zhang Y, Qiu X, et al. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. Sci Rep. 2015;5:8096.

(28) Spencer MD, Hamp TJ, Reid RW, Fischer LM, Zeisel SH, Fodor AA. Association between composition of the human gastrointestinal microbiome and development of fatty liver with choline deficiency. Gastroenterology. 2011;140:976-86.

(29) Engstler AJ, Aumiller T, Degen C, Dürr M, Weiss E, Maier IB, et al. Insulin resistance alters hepatic ethanol metabolism: studies in mice and children with non-alcoholic fatty liver disease. Gut. 2015 May 25 [Epub ahead of print].

(30) Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature. 2012;482:179-85.

(31) Sawada K, Ohtake T, Hasebe T, Abe M, Tanaka H, Ikuta K, et al. Augmented hepatic toll-like receptors by fatty acids trigger the pro-inflammatory state of non-alcoholic fatty liver disease in mice. Hepatol Res. 2014;44:920-34.

(32) Wagnerberger S, Spruss A, Kanuri G, Volynets V, Stahl C, Bischoff SC, et al. Toll-like receptors 1-9 are elevated in livers with fructose-induced hepatic steatosis. Br J Nutr. 2012;107:1727-38.

(33) Miura K, Ohnishi H. Role of gut microbiota and toll-like receptors in nonalcoholic fatty liver disease. World J Gastroenterol. 2014;20:7381-91.

(34) Seki E, Schnabl B. Role of innate immunity and the microbiota in liver fibrosis: crosstalk between the liver and gut. J Physiol. 2012;590:447-58.

(35) Jiang C, Xie C, Li F, Zhang L, Nichols RG, Krausz KW, et al. Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. J Clin Invest. 2015;125:386-402.

(36) Lirussi F, Mastropasqua E, Orando S, Orlando R. Probiotics for non-alcoholic fatty liver disease and/or steatohepatitis. Cochrane Database Syst Rev. 2007;(1):CD005165

(37) Tarantino G, Finelli C. Systematic review on intervention with prebiotics/probiotics in patients with obesity-related nonalcoholic fatty liver disease. Future Microbiol. 2015;10:889-902.

LATEST ADVANCES IN THE USE OF PROBIOTICS/SYNBIOTICS FOR ACUTE INFECTIOUS DIARRHEA IN CHILDREN

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INTRODUCTION

Acute diarrhea is defined by the World Health Organization as the passage of three or more loose or liquid stools per day, for three or more days and for less than 14 days; it continues to be a leading cause of morbidity, hospitalization and mortality worldwide, resulting in a huge financial burden [1, 2]. There are an estimated 1.7 billion episodes of diarrhea annually and it is one of the leading causes of death from infectious diseases in children worldwide, accounting for approximately 9% off all mortality in children under 5 years old [2, 3]. A marked decrease in mortality due to diarrhea (~54% reduction) has been observed between 2000 and 2013 [2]. Despite this significant achievement, diarrhea is still a major cause of mortality in developing countries and it is a leading cause of emergency department visits and hospitalizations in developed countries [1]. Major etiological causes of acute infectious diarrhea in children in developed countries are viruses, in particular rotavirus. Prior to the introduction of rotavirus vaccines in 2006, rotavirus was the leading cause of severe gastroenteritis among European children under the age of five. After the introduction of rotavirus vaccines in routine immunization program, the incidence of severe rotavirus gastroenteritis, hospitalization and deaths due to sever gastroenteritis significantly decreased. Across Europe, the effectiveness of the rotavirus vaccine in reducing rotavirus-related use of healthcare services ranged from 68% to 98% and reductions in the number of hospitalizations due to rotavirus infections have been observed in Europe, the United States and Latin America [4]. Norovirus is another worldwide pathogen responsible for causing acute diarrhea in all age groups. In Europe, norovirus infection may cause up to 5.7 million illnesses in the community, 800,000 medical visits, 53,000 hospitalizations and 102 deaths every year in children under the age of five [5]. In developing parts of the world, including Africa and Asia, most cases of diarrhea are due to rotavirus, Cryptosporidium, Shigella, and heat-stable toxin-producing enterotoxigenic Escherichia coli, according to results from the Global Enteric Multicenter Study [6].

The main treatment for all children with dehydration as a consequence of diarrhea is the administration of oral rehydration solution (ORS). The widespread routine use of ORS in children has led to a significant decrease in mortality [7, 8]. However, water and electrolyte replacement does not substantially shorten the frequency or duration of diarrhea and has not been found to reduce stool volume, prompting a growing interest in adjunctive treatments [8].

Probiotics are live microorganisms that confer a health benefit to the host, when administered in appropriate amounts [9]. All published studies, meta-analysis and guidelines have shown that the effects of probiotic on acute infectious diarrhea are strain specific, and it is not possible to extrapolate the positive or negative results of one probiotic/symbiotic preparation to others [8-11]. Probiotics have been proposed as a complementary therapy in the treatment of acute diarrhea [7], with improvements in the duration and severity of diarrhea and the duration of hospitalization reported with probiotics. There have been many randomized-controlled studies and observational studies with different probiotics and synbiotic preparations, and well-conducted meta-analyses are also available. The majority of the published studies revealed that probiotics can reduce the duration of diarrhea by approximately one day, shorten the initial phase of watery stools, and decrease the length of hospital stay [7, 8-12]. In this report, the current situation and a clinical update of the benefits of probiotics and synbiotics in patients with acute diarrhea are presented.

Lactobacillus rhamnosus GG

Lactobacillus rhamnosus GG is one of the well-studied probiotics in patients with acute infectious diarrhea. In 2013, Szajewska and colleagues published a systematic review examining the use *L. rhamnosus GG* as a single strain in combination with oral and/or intravenous rehydration therapy in 15 studies, with a total of 2,963 in- or outpatients [13]. The daily doses of *L. rhamnosus GG* ranged from 1.2×10^8 colony-forming units (CFU) to 2×10^{12} CFU and 11 randomized clinical trials including 2,444 children showed that *L. rhamnosus GG* reduced the duration of diarrhea by approximately one day and was more effective at doses $\geq10^{10}$ CFU/day than at lower doses. A meta-analysis of four randomized controlled trials also showed a reduction of approximately 0.8 day in duration of hospitalization for those treated with *L. rhamnosus GG* [13].

Saccharomyces boulardii CNCM I-745

The clinical effects of *Saccharomyces boulardii* CNCM I-745 on acute infectious diarrhea have been extensively studied within different settings, in both developing and developed countries [12, 14]. There is strong evidence that this probiotic offers clinically significant benefits no matter what the cause of the gastroenteritis is (viral, bacterial, protozoan), in both developed and developing countries. According to 11 randomized controlled trials (total number of children, N=1306, *S. boulardii* CNCM I-745 group, n=651; control group, n=655), *S. boulardii* CNCM I-745 significantly reduced the duration of diarrhea by approximately 24 hours. *S. boulardii* CNCM I-745 also reduced the duration of hospitalization by approximately 20 hours. Based on the results of nine randomized controlled trials involving 1,128 children, *S. boulardii* CNCM I-745 significantly reduced the risk of diarrhea by 48% on the third day after the start of treatment [12]. *S. boulardii* is safe in children with acute diarrhea, and no adverse event associated with *S. boulardii* were reported in these studies [12]. In addition to the reduction of duration of diarrhea and hospitalization, there are some promising end points regarding the use of *S. boulardii* in acute infectious diarrhea, such as the reduction of persistent diarrhea lasting >7 days, the reduction in the recurrence of new episodes of diarrhea, and the reduction of mean total ORS volume used [15-17]. In adult patients with giardiasis and pediatric patients with amebiasis *S. boulardii* CNCM I-745 with metronidazole has positive effects on the resolution of diarrhea as well as on the clearance of *Giardia* or *Entamoeba* cysts [18-19]. Our recent multicenter, randomized, prospective, controlled, single blind, clinical trial included 363 children with acute watery diarrhea and aimed to assess the effect of *S. boulardii* CNCM I-745 in hospitalized children, children requiring emergency care unit (ECU) stay, and outpatient settings [20].

The duration of diarrhea was approximately 24 hours shorter in the *S. boulardii*-treated group compared with the placebo group (75.4±33.1 vs. 99.8±32.5 hours, p<0.001; Figure 1). The effect of *S. boulardii* (diarrhea-free children) was observed starting at 48 hours. After 72 hours, only 27.3% of the children receiving probiotics still had watery diarrhea, in contrast to 48.5% in the control group (p<0.001; Figure 2). The duration of diarrhea was significantly reduced in the probiotic group in hospital, ECU and outpatient settings (p<0.001, p<0.01 and p<0.001, respectively; Figure 1). The mean length of hospital stay was shorter by more than 36 hours and the mean length of ECU stay was shorter by more than 19 hours in the *S. boulardii* group compared with the control group (p<0.001). This is the first study showing the effects of *S. boulardii* on acute infectious diarrhea in emergency care unit [20].

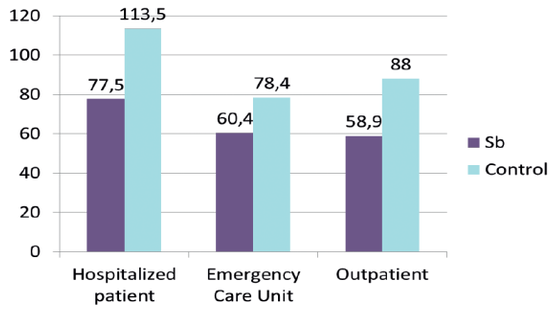
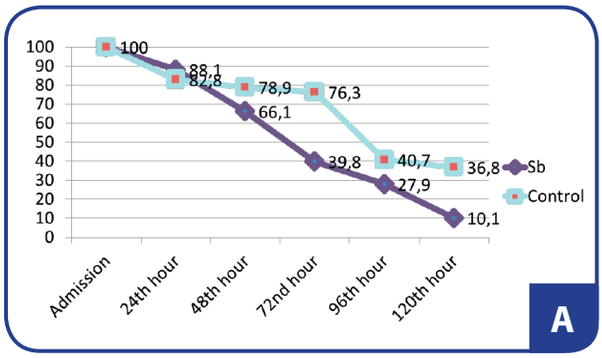
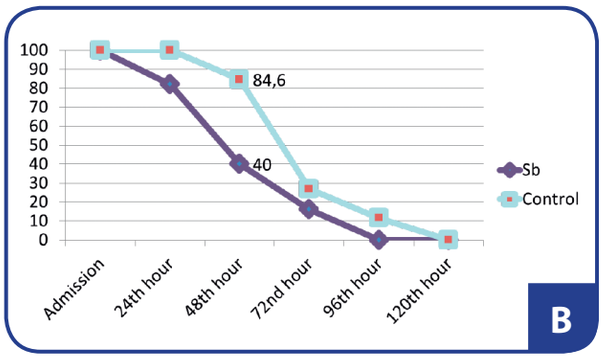


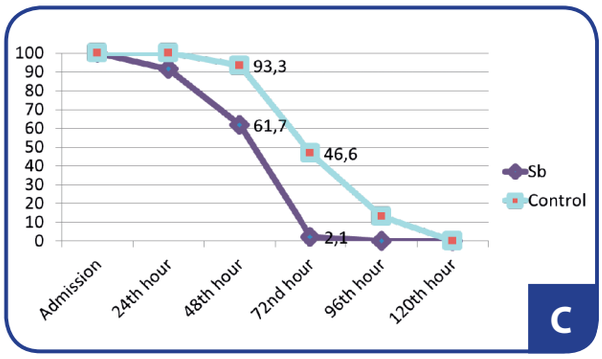
Figure 1.
Duration of diarrhea in hours in Saccharomyces boulardii CNCM I-745-treated groups versus control groups in hospital, emergency care unit and outpatient settings [20]. Sb, S. boulardii CNCM I-745-treated groups.

Figure 2.

Percentage of children with diarrhea in Saccharomyces boulardii CNCM I-745 versus control groups during 5 days in hospital (A), emergency care unit (B) and outpatient setting (C). Sb, S. boulardii CNCM I-745-treated groups.





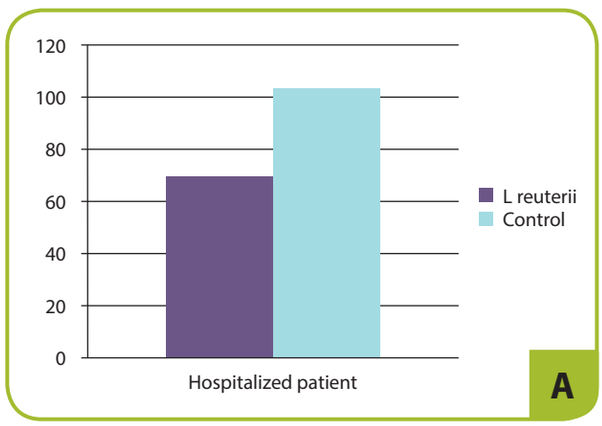


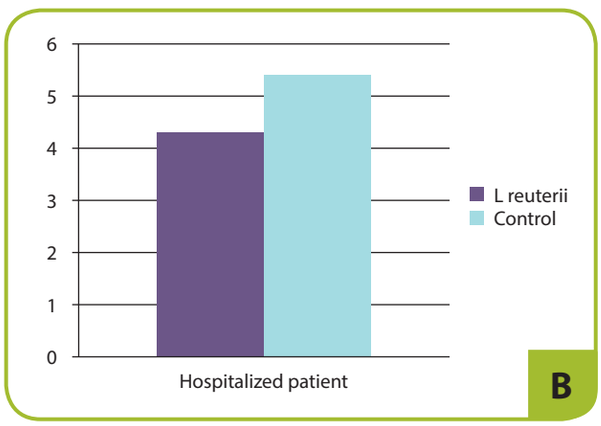
Lactobacillus reuteri DSM 17938

Three randomized controlled clinical trials evaluated the effects of *Lactobacillus reuteri* DSM 17938 [21-23]; two of these studies were performed in hospitalized children and one in ambulatory patients. Meta-analysis of the two studies with hospitalized children revealed that children treated with *L. reuteri* DSM 17938 had a significant reduction in the duration of diarrhea of 32 hours. In hospitalized children with acute gastroenteritis, addition of *L. reuteri* DSM 17938 to standard rehydration therapy increased the chance of cure on day 3 and reduced the risk of watery diarrhea on day 2 to day 4 of intervention [24]. Our hospital-based clinical study showed that *L. reuteri* DSM 17938, reduced the duration of hospitalization by approximately 1.15 days (Figure 3) [21]. Our recent trial is the first multicenter, randomized, single-blinded, case control clinical trial to examine the efficacy of *L. reuteri* DSM 17938 (1×108 CFU daily for 5 days) in outpatient children with acute infectious diarrhea [22]. In outpatient setting, the mean duration of diarrhea was significantly reduced by approximately 15 hours in the *L. reuteri* DSM 17938 group. The percentage of children with diarrhea was lower in the *L. reuteri* DSM 17938 group (44.8%) after 48 hours than in the controls group (87%; $p<0.01$) [22]. No adverse effects related to *L. reuteri* DSM 17938 were noted amongst both inpatients and outpatient settings [21, 22, 24].

Figure 3.

Duration of diarrhea in hours (A) and length of hospital stay in days (B) in children receiving L. reuterii DSM 17938 (L reuterii) versus patients receiving placebo (Control) [21].





SYNBIOTICS

Synbiotics, mixtures of probiotics and prebiotics beneficially affect the host and improve his/her welfare by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract and selectively stimulating the growth, and/or activating the metabolism of one or a limited number of health-promoting bacteria [9]. Clinical studies focusing on the effects of synbiotics on acute infectious diarrhea are limited and mainly performed on different preparations [7]. Current ESPID/ESPGHAN guidelines mention that none of the synbiotics studied thus far can be recommended until confirmatory data are available, and so far, the effects have only been seen in one clinical study for each combination [7]. Two studies, one conducted in Belgium (combination of *Streptococcus thermophilus, L. rhamnosus, L. acidophilus, Bifidobacterium lactis, B. infantis* and fructooligosaccharides) and one conducted in Italy (*L. paracasei B21060* in combination with arabinogalactan and xylooligosaccharides), also showed promising results regarding the effects of synbiotics on acute infectious diarrhea [25, 26]. Our previous study, which used a different synbiotic preparation with a daily dose of 2.5×10^9 CFU live bacteria including *L. acidophilus, L. rhamnosus, B. bifidum, B. longum, Enterococcus faecium,* and 625 mg fructooligosaccharide for 5 days, showed a significant reduction in the duration of diarrhea of approximately 36 hours in children with acute infectious diarrhea. The effect started 24 hours after the first dose was administered, and a greater effect was been seen at the 48th and 72nd hour following the start of the intervention [27]. Two recent studies from Turkey have examined the efficacy of a combination of *B. lactis* B94 and inulin in the treatment of acute infectious diarrhea in children [28, 29]. In the first study, Islek and colleagues enrolled 156 children aged between two and 60 months with acute diarrhea; some patients received *B. lactis* B94 (5×10^{10} CFU) plus 900 mg inulin while others received placebo [28]. The duration of diarrhea was significantly reduced (by an average of 31 hours) and the number of diarrheal stools on the third day was significantly lower in the synbiotic group, especially in children with acute diarrhea due to rotavirus [28]. The second study was a prospective, multicenter, randomized, double blind, clinical trial, which had 80 children enrolled, aged three to 60 months, with acute watery diarrhea [29]. Children received oral rehydration with *B. lactis* ($5x10^9$ CFU) plus inulin (900 mg), *B. lactis* alone ($5x10^9$ CFU), inulin (900 mg) or placebo for 5 days. The duration of diarrhea was significantly reduced in the *B. lactis* plus inulin and *B. lactis* alone groups compared with the inulin alone and placebo groups ($p<0.001$), while there were no statistical difference between the *B. lactis* plus inulin and *B. lactis* alone groups. After 72 hours, the percentage of diarrhea-free children was significantly larger in the *B. lactis* plus inulin and *B. lactis* alone groups than in the other groups. Mean length of hospital stay was approximately 24–30 hours lower in the *B. lactis* plus inulin and *B. lactis* alone groups compared with the placebo group. In summary, these results show that *B. lactis* plus inulin and *B. lactis* alone reduce the duration of diarrhea in the same way and that inulin alone has no effect on the duration of diarrhea [29]. The efficacy of synbiotic preparations that contain prebiotics should be assessed by comparing the effects of the same preparations with or without prebiotics in children with acute infectious diarrhea. Further studies investigating the same synbiotic preparation in different clinical settings will allow clinicians to make better informed recommendations.

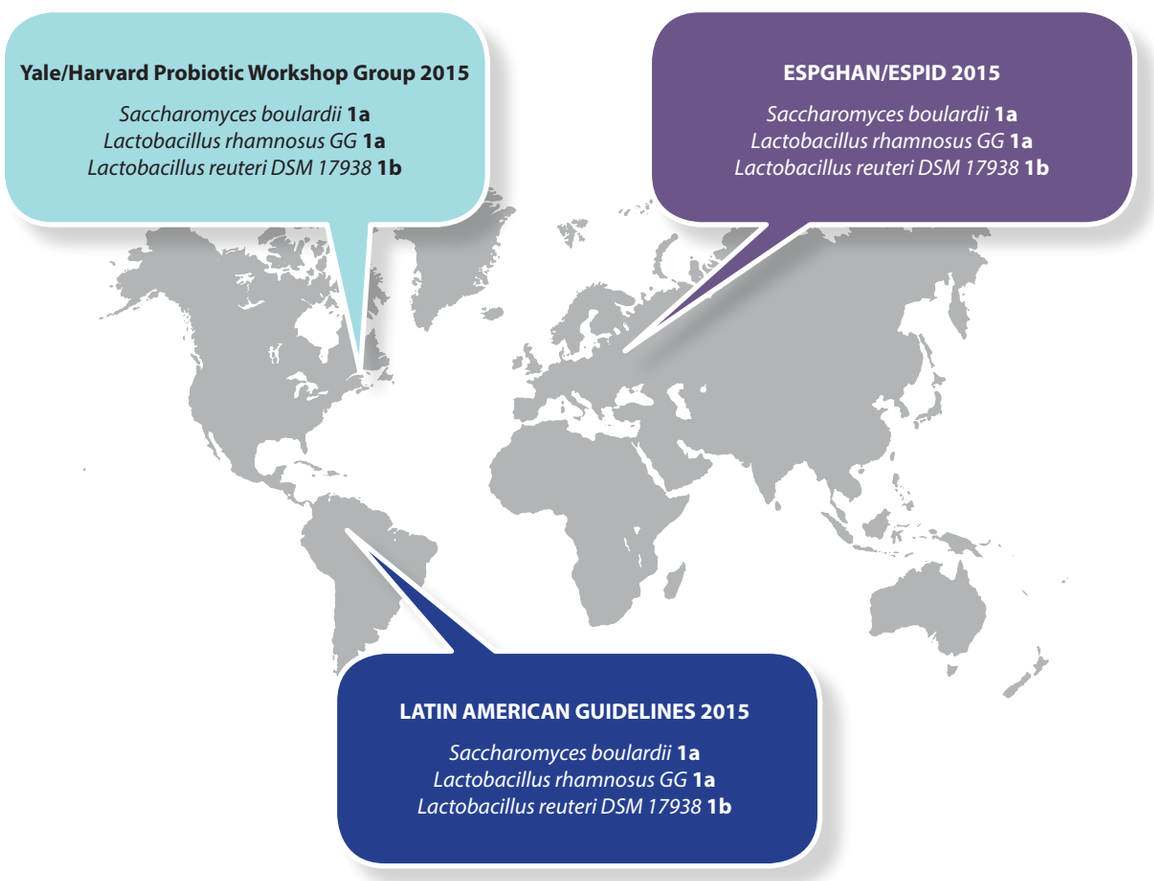
COST-EFFECTIVENESS OF USING PROBIOTICS IN CHILDREN WITH ACUTE INFECTIOUS DIARRHEA

The benefit of using probiotics/synbiotics in the management of acute infectious diarrhea to reduce the duration of diarrhea by approximately 1 day, often raises the question of the cost-effectiveness of these interventions. Acute infectious diarrhea is a substantial financial burden for the families of affected children and the healthcare system. We used our previous clinical data with *S. boulardii* CNCM I-745 to calculate the direct cost associated with treating children with acute infectious diarrhea in ambulatory care, emergency care unit and in hospitalized children and extrapolated these data to the number of all cases of rotavirus-induced in a year. In hospitalized children, *S. boulardii* CNCM I-745 significantly reduced the cost of hospitalization ($p<0.001$) and as emergency unit stay was reduced by 19 hours, the direct cost significantly lowered ($p<0.001$). When we extrapolated these findings to the yearly number of rotavirus gastroenteritis in Turkey, the total cost associated with hospitalization and emergency care unit stay was reduced by 25% or US$51 per patient. Over a year, if *S. boulardii* was administered to all rotavirus cases of children under 5 years old, the total cost would decrease by 23% or US$11.3 per patient. This study is the first to address the economic implications of using probiotics, more specifically *S. boulardii* CNCM I-745, to treat children hospitalized with acute diarrhea [30].

CURRENT RECOMMENDATIONS AND GUIDELINES

Since 2014, three important guidelines, including those from the European Society for Paediatric Gastroenterology, Hepatology and Nutrition and the European Society of Paediatric Infectious Diseases (ESPGHAN/ESPID), guidelines from Latin American (LATAM) experts and Guidelines from the Yale and Harvard Probiotic Workshop Group have been published or revised to encompass the use of probiotics (Figure 4) [7, 31, 32].

Figure 4. **Current recommendations and guidelines**



In 2014, ESPGHAN/ESPID published their current recommendations in children with acute gastroenteritis, a revised version of their first recommendations published in 2008 [7].
They highlighted that probiotics used as adjunct to ORS reduced the duration of diarrhea by approximately one day; however, the effects of probiotic are strain-specific, and efficacy and safety profiles should be established for each strain. Moreover, the safety and clinical efficacy of one probiotic microorganism should not be extrapolated to other probiotic microorganisms. Selected probiotics can be used in children with acute gastroenteritis (recommendation at A1 level). According to this guideline, *S. boulardii* and *L. rhamnosus GG* may be considered in the management of children with acute gastroenteritis as an adjunct to rehydration therapy as A-1 level of evidence (low-quality evidence, strong recommendation). *L. reuteri* DSM 17938 was also a recommended strain (weak recommendation, very low-quality evidence). Another heat-killed Lactobacillus strain (*L. acidophilus LB*) also have some efficacy in reducing diarrhea related symptoms in children (weak recommendation, very low-quality evidence) [7].
According to the 2014 guidelines, none of the synbiotics can be recommended (II, B [weak recommendation, low-quality evidence]). ESPGHAN/ESPID guidelines also strongly recommended against the administration of *E. faecium* strain SF68 because of the risk of spreading plasmids carrying vancomycin resistance [7-10].

The consensus opinion of the participants in the 4th Triennial Yale/Harvard Workshop on Probiotic Recommendations was published in November 2015. It highlighted the benefits of using *L. rhamnosus GG, S. boulardii* and *L. reuteri* for the treatment of children with acute infectious diarrhea [31].

The LATAM Guidelines, published in 2015, consider that a decrease in the duration of diarrhea, as well as hospitalization length of children with acute infectious diarrhea, is an important benefit from a social and economic development point of view. They recommended a priority of 1a for *L. rhamnosus GG* and *S. boulardii,* and a priority of 1b for *L. reuteri* for the treatment of acute infectious diarrhea [32].

PROBIOTICS/SYNBIOTICS USE IN PATIENTS WITH ACUTE INFECTIOUS DIARRHEA: FUTURE PROSPECTS

- Probiotics are widely studied therapeutic options for acute infectious diarrhea but their effects are strain-specific. All new strains or combinations of strains should be assessed for the treatment of acute infectious diarrhea before widespread use.
- After the routine use of rotavirus vaccine, the seroepidemiology of acute infectious diarrhea changed and the effect of probiotics should be specifically evaluated in these geographical regions.
- Norovirus is one of the leading causes of acute infectious diarrhea in children and adults, and the effects of probiotics on acute infectious diarrhea due to norovirus, should be investigated.
- Several mechanisms of action have been reported or proposed regarding the effects of probiotics on acute diarrhea. However, there are limited data available on the effects probiotics have on the composition of the gut microbiota. An ongoing clinical study aims to examine the potential effects probiotics have on the composition of the gut microbiota in children with rotavirus-induced acute infectious diarrhea.
- While there are numerous clinical studies and evidence-based guidelines available, most healthcare professionals only have a limited understanding of the use of probiotics for the treatment of acute infectious diarrhea in children. Scientific organizations with a focus on probiotics and/or microbiota should contribute to promoting and delivering professional development for healthcare professionals on the clinical applications of probiotics, prebiotics and synbiotics.

References

(1) Walker CL, Rudan I, Liu L, Nair H, Theodoratou E, Bhutta ZA, et al. Global burden of childhood pneumonia and diarrhoea. Lancet. 2013;381:1405-16.

(2) Leung DT, Chisti MJ, Pavia AT. Prevention and control of childhood pneumonia and diarrhea. Pediatr Clin North Am. 2016;63:67-79.

(3) Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015;385:430-40.

(4) Karafillakis E, Hassounah S, Atchison C. Effectiveness and impact of rotavirus vaccines in Europe, 2006-2014. Vaccine. 2015;33:2097-107.

(5) Kowalzik F, Riera-Montes M, Verstraeten T, Zepp F. The burden of norovirus disease in children in the European Union. Pediatr Infect Dis J. 2015;34:229-34.

(6) Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet. 2013;382:209-22.

(7) Guarino A, Ashkenazi S, Gendrel D, Lo Vecchio A, Shamir R, Szajewska H. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition/ European Society for Pediatric Infectious Diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe: update 2014. J Pediatr Gastroenterol Nutr. 2014;59:132-52.

(8) Vandenplas Y, Salvatore S, Vieira M, Devreker T, Hauser B. Probiotics in infectious diarrhoea in children: are they indicated? Eur J Pediatr. 2007;166:1211-8.

(9) Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506-14.

(10) Szajewska H, Guarino A, Hojsak I, Indrio F, Kolacek S, Shamir R, et al. Use of probiotics for management of acute gastroenteritis: a position paper by the ESPGHAN Working Group for Probiotics and Prebiotics. J Pediatr Gastroenterol Nutr. 2014;58:531-9.

(11) Pieścik-Lech M, Shamir R, Guarino A, Szajewska H. Review article: the management of acute gastroenteritis in children. Aliment Pharmacol Ther. 2013;37:289-303.

(12) Dinleyici EC, Eren M, Ozen M, Yargic ZA, Vandenplas Y. Effectiveness and safety of Saccharomyces boulardii for acute infectious diarrhea. Expert Opin Biol Ther. 2012;12:395-410.

(13) Szajewska H, Skórka A, Ruszczyński M, Gieruszczak-Białek D. Meta-analysis: Lactobacillus GG for treating acute gastroenteritis in children--updated analysis of randomised controlled trials. Aliment Pharmacol Ther. 2013;38:467-76.

(14) Dinleyici EC, Kara A, Ozen M, Vandenplas Y. Saccharomyces boulardii CNCM I-745 in different clinical conditions. Expert Opin Biol Ther. 2014;14:1593-609.

(15) Billoo AG, Memon MA, Khaskheli SA, Murtaza G, Iqbal K, Saeed Shekhani M, et al. Role of a probiotic (Saccharomyces boulardii) in management and prevention of diarrhoea. World J Gastroenterol. 2006;12:4557-60.

(16) Villarruel G, Rubio DM, Lopez F, Cintioni J, Gurevech R, Romero G, et al. Saccharomyces boulardii in acute childhood diarrhoea: a randomized, placebo-controlled study. Acta Paediatr. 2007;96:538-41.

(17) Riaz M, Alam S, Malik A, Ali SM. Efficacy and safety of Saccharomyces boulardii in acute childhood diarrhea: a double blind randomised controlled trial. Indian J Pediatr. 2012;79:478-82

(18) Besirbellioglu BA, Ulcay A, Can M, Erdem H, Tanyuksel M, Avci IY, et al. Saccharomyces boulardii and infection due to Giardia lamblia. Scand J Infect Dis. 2006;38:479-81.

(19) Dinleyici EC, Eren M, Yargic ZA, Dogan N, Vandenplas Y. Clinical efficacy of Saccharomyces boulardii and metronidazole compared to metronidazole alone in children with acute bloody diarrhea caused by amebiasis: a prospective, randomized, open label study. Am J Trop Med Hyg. 2009;80:953-5.

(20) Dinleyici EC, Kara A, Dalgic N, Kurugol Z, Arica V, Metin O, et al. Saccharomyces boulardii CNCM I-745 reduces the duration of diarrhoea, length of emergency care and hospital stay in children with acute diarrhoea. Benef Microbes. 2015;6:415-21.

(21) Dinleyici EC; PROBAGE Study Group, Vandenplas Y. Lactobacillus reuteri DSM 17938 effectively reduces the duration of acute diarrhoea in hospitalised children. Acta Paediatr. 2014;103:e300-5.

(22) Dinleyici EC, Dalgic N, Guven S, Metin O, Yasa O, Kurugol Z, et al. Lactobacillus reuteri DSM 17938 shortens acute infectious diarrhea in a pediatric outpatient setting. J Pediatr (Rio J). 2015;91:392-6.

(23) Francavilla R, Lionetti E, Castellaneta S, Ciruzzi F, Indrio F, Masciale A, et al. Randomised clinical trial: Lactobacillus reuteri DSM 17938 vs. placebo in children with acute diarrhoea—a double-blind study. Aliment Pharmacol Ther. 2012;36:363-9.

(24) Szajewska H, Urbańska M, Chmielewska A, Weizman Z, Shamir R. Meta-analysis: Lactobacillus reuteri strain DSM 17938 (and the original strain ATCC 55730) for treating acute gastroenteritis in children. Benef Microbes. 2014;5:285-93.

(25) Passariello A, Terrin G, Cecere G, Micillo M, De Marco G, Di Costanzo M, et al. Randomised clinical trial: efficacy of a new synbiotic formulation containing Lactobacillus paracasei B21060 plus arabinogalactan and xilooligosaccharides in children with acute diarrhoea. Aliment Pharm Ther. 2012;35:782-8.

(26) Vandenplas Y, De Hert SG; PROBIOTICAL-study group. Randomised clinical trial: the synbiotic food supplement Probiotical vs. placebo for acute gastroenteritis in children. Aliment Pharmacol Ther. 2011;34:862-7.

(27) Dinleyici EC, Dalgic N, Guven S, Ozen M, Kara A, Arica V, et al. The effect of a multispecies synbiotic mixture on the duration of diarrhea and length of hospital stay in children with acute diarrhea in Turkey: single blinded randomized study. Eur J Pediatr. 2013;172:459-64.

(28) İşlek A, Sayar E, Yılmaz A, Baysan BÖ, Mutlu D, Artan R. The role of Bifidobacterium lactis B94 plus inulin in the treatment of acute infectious diarrhea in children. Turk J Gastroenterol. 2014;25:628-33.

(29) Dinleyici EC, Kurugol Z, Dalgic N, Yasa O, Guven S, Nalbantoglu B, et al. The effects of Bifidobacterium lactis, Bifidobacterium lactis plus inulin, inulin and placebo on the duration of diarrhea in children: a randomised, multi-center, double blind, placebo controlled clinical trial. ESPGHAN 48th Annual Meeting of the European Society for Paediatric Gastroenterology Hepatology and Nutrition, 6-9 May 2015, Amsterdam, PO-G-0180.

(30) Dinleyici EC, Kara A, Ozen M, Dalgic N, Kurugol Z, Guven S, et al. Cost-effectiveness analysis of add-on Saccharomyces boulardii CNCM I-745 in children with acute infectious diarrhea in Turkey (PROBAGE Study). ESPGHAN 48th Annual Meeting of the European Society for Paediatric Gastroenterology Hepatology and Nutrition, 6-9 May 2015, Amsterdam, PO-G-0183.

(31) Floch MH, Walker WA, Sanders ME, Nieuwdorp M, Kim AS, et al. Recommendations for probiotic use-2015 update: proceedings and consensus opinion. J Clin Gastroenterol. 2015;49(Suppl 1):S69-73.

(32) Cruchet S, Furnes R, Maruy A, Hebel E, Palacios J, Medina F, et al. The use of probiotics in pediatric gastroenterology: a review of the literature and recommendations by Latin-American experts. Paediatr Drugs. 201517:199-216.

PROBIOTIC THERAPY IN IRRITABLE BOWEL SYNDROME

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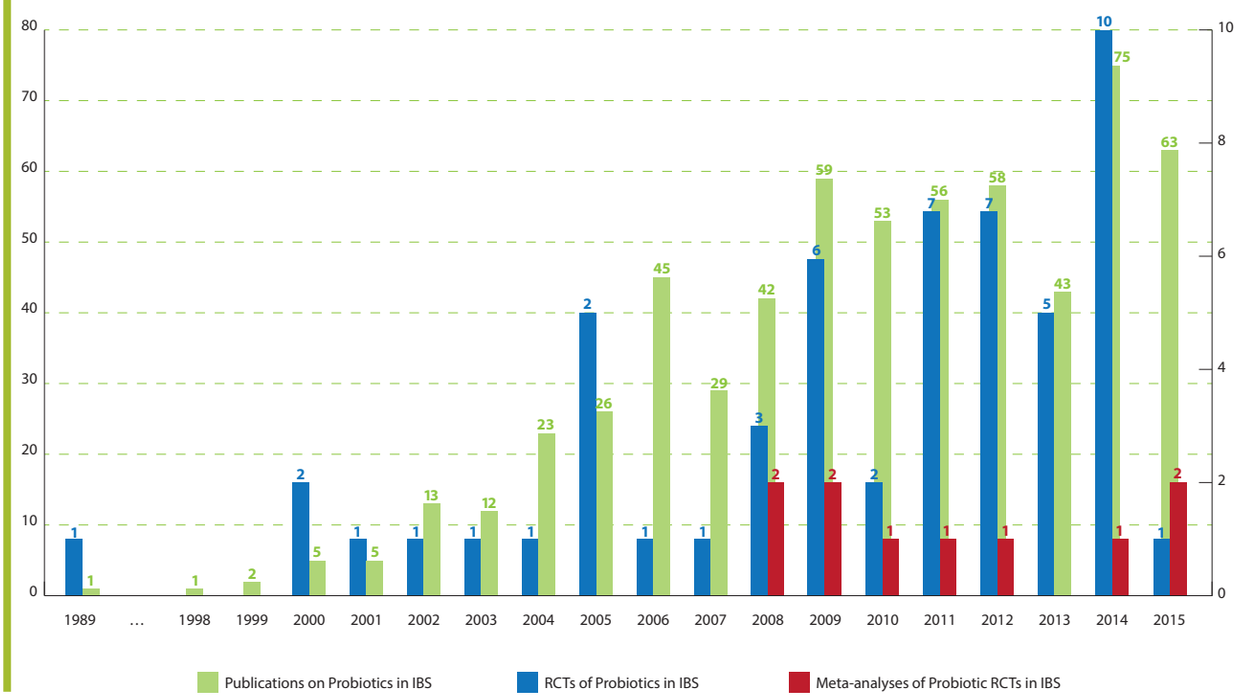
BACKGROUND

A recent systematic review analyzed the results of 56 randomized controlled trials (RCTs) that investigated the efficacy of probiotic preparations, compared with placebo, for the treatment of patients with irritable bowel syndrome (IBS) [1-56], between 1989 and 2015 and that have been either included or excluded from 9 meta-analyses [57-65] published between 2008 and 2015 (Figure 1). Concerns were raised [66] regarding the substantiality of the evidence to support the efficacy of the probiotic preparations, and the quality of both the RCTs and the meta-analyses. This paper summarizes the review and addresses critical questions relevant to doctors who are faced with the difficulty of treating patients with IBS.

Figure 1. *Number of publications per year on the role of probiotics in IBS (left Y-axis), number of randomized and controlled trials (RCT) of probiotics in IBS (right Y-axis), and number of meta-analyses of probiotic RCT in IBS (right Y-axis)*



Living microorganisms from dairy products have been used for centuries to modify bowel functions, treat bowel disorders, or to improve general health; however, they only received the designation "probiotics", meaning "supporting life", in the 50s'. The modern definition adopted by the International Scientific Association for Probiotics and Prebiotics (ISAPP) describes probiotics as living organisms that, when ingested in sufficient amounts, may be beneficial for the host [67]. It evades any speculations regarding the possible mechanisms of action, as concepts have changed substantially during the last few decades with the development of modern laboratory techniques and the replacement, to some extent, of culture technology by molecular analyses, and with growing understanding of the interactions between the human body and these microorganisms [68].

There has been a growing interest in probiotic research and the use of probiotics as treatments over the last 20 years; indeed, only nine scientific articles were published in 1995 whilst over 1,500 articles were published in 2014, as found by searching the PubMed library using the term "probiotics" alone. One of the reasons behind the sudden interest in probiotics may be associated with the concomitant growing awareness of so-called functional gastrointestinal (GI) disorders, such as IBS, functional dyspepsia, functional constipation, etc [69]. These disorders have a high impact on the quality of life of affected patients and have become a major economic burden for society; furthermore, patients do not usually respond favorably to conventional treatment strategies, including novel and traditional medications such as laxatives, bulking agents, and dietary interventions [70]. Probiotics appear to offer a low-cost alternative with a high safety margin. Among the disorders that have been shown to benefit from probiotic treatment in placebo-controlled, double-blinded, randomized studies, are antibiotic-associated diarrhea [71], traveler's diarrhea [72], rota-virus-induced diarrhea [73], and *Clostridium difficile*-associated diarrhea [74]. Beneficial effects have also been shown in diarrhea in children [75]. Some bacterial strains such as *Escherichia coli Nissle* showed efficacy in relieving chronic constipation [76] and in the maintenance of remission in ulcerative colitis [77].

IBS clinically manifests with abdominal pain associated with other GI symptoms like constipation, diarrhea, bloating, and flatulence in the absence of any organic cause for the symptoms; it is one of the most frequent disorders in the general population and in private practice, as reported by primary care physicians and gastroenterologists [78]. Across 42 countries worldwide, the prevalence is 11.2% [79]. The pathophysiology of IBS is believed to be a consequence of the interplay between gut-brain axis disturbances, local immune changes and inflammation in the gut wall, modifications in gut permeability, and in the composition of gut microorganisms. Consequently, probiotics are regarded as putative agents that could possibly affect several of these targets and therefore enhance remission in chronic and recurrent conditions. Some hypotheses have been put forward regarding how probiotics may influence brain functions [80] and it has been suggested that they may improve central functions via stress-moderating effects [81].

In this paper we will analyze the current evidence to support the use of probiotics in the treatment of IBS and attempt to answer some questions relevant to the day to day clinical management of these patients.

SCIENTIFIC PERSPECTIVE

The highest level of evidence (evidence level 1a) of the efficacy of a specific clinical intervention can be drawn from meta-analyses of RCTs. An RCT is a study designed to allow testing of a therapeutic agent against a placebo in which neither participants nor researchers know what substance (drug or placebo) is being given to a participant. Although the number of participants in each RCT is usually limited by time and financial constraints, meta-analyses allow to conduct statistical analyses of data from smaller studies and to build an overview of treatment efficacy.

During the past 15 years, nine meta-analyses of RCTs on the efficacy of probiotic therapy in the treatment of IBS were published [57-65]. Prior to statistical analysis, a literature search was performed with entry criteria specific to the meta-analysis and those studies meeting the allocated criteria were selected from the body of literature, as required by meta-analysis methodology [82].

As the body of studies published during this period increased, it is not surprising that, with each subsequent meta-analysis, so did the total number of studies available. However, the selection of studies included was not consistent from one meta-analysis to another and many studies that were included in a meta-analysis were excluded from another. Amongst the 56 studies that we identified for a recent systematic review [66], only a minority were found to be acceptable (Figure 2).

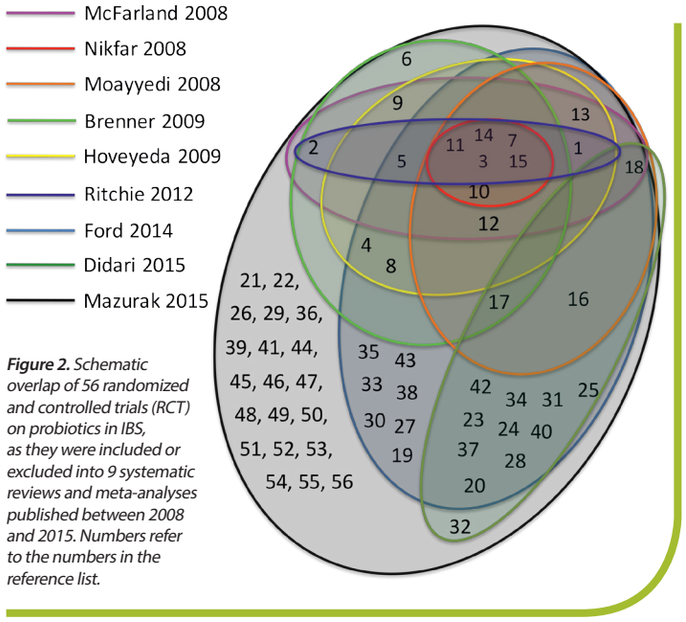


Figure 2. *Schematic overlap of 56 randomized and controlled trials (RCT) on probiotics in IBS, as they were included or excluded into 9 systematic reviews and meta-analyses published between 2008 and 2015. Numbers refer to the numbers in the reference list.*

Despite this heterogeneity across meta-analyses, they all converge in their conclusions:

- «Probiotics may improve symptoms of IBS and can be used as supplement to standard therapy» [57].
- «While our analyses suggest that probiotic use may be associated with improvement in IBS symptoms compared to placebo, these results should be interpreted with caution given the methodological limitations of contributing studies. Probiotics warrant further study as a potential therapy for IBS» [58].
- «Probiotics may have a role in alleviating some of the symptoms of IBS; however further research should focus on the type, optimal dose of probiotics and the subgroups of patients who are likely to benefit the most» [60].
- «Six of the eight diseases (…irritable bowel syndrome…) showed positive significant effects.... Across all diseases and probiotic species, positive significant effects of probiotics were observed for all age groups, single vs multiple species, and treatment lengths» [63].
- «Probiotics are effective treatments for IBS, although which individual species and strains are the most beneficial remains unclear» [64].

The reason why these conclusions have been written with caution is inherent to the nature of meta-analyses; to provide a reliable result, the studies selected for the statistical analyses should provide homogeneous data, such as patient inclusion and exclusion criteria, measures of primary outcome, treatment agent, dosage, population studied, duration of treatment, and so on. This implies very strict inclusion and exclusion criteria for all studies, and even then, as demonstrated by Figure 2, there is no guaranty that all meta-analyses will integrate the same combination of studies.

The other reason for why these meta-analyses have not provided reliable information is the nature of the probiotics themselves. Generally, microorganisms may be beneficial for the host but there may be substantial differences between different species, and the number of known microorganisms that may have beneficial effects has dramatically grown in the past few years, due to more cost-effective genetic methods available for species identification.

Many of the 56 RCT assessments of probiotics in IBS have used a whole range of different probiotics, and sometimes two or more strains/species were compared in a single meta-analysis; such a comparison would not be easily acceptable in drug studies, e.g. it is analogous to comparing antidepressants of different kind in a single meta-analysis, without considering their mechanism of action. Finally, producers of probiotics have accounted for potential differences in single strains by sometimes mixing over 10 different strains, with different dosages in order to increase the likelihood of effectiveness in RCTs.

With all these considerations, we decided to perform a systematic review as opposed to a meta-analysis. We chose pre-defined criteria for paper selection and reviewed the efficacy of probiotics in the management of IBS, without using a statistical method. This approach allowed us to integrate 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 valuable information on the efficacy of probiotics in patients with IBS.

MULTI-STRAIN PREPARATIONS

Half of the selected studies consisted of trials with probiotic preparations that contained multiple strains, often from different species. The idea behind multi-strain or "multi-species" preparations is that probiotic mixtures may provide a better chance of survival of exogenous bacteria in the GI tract, but also that different microorganisms may develop a synergistic action that will enhance the beneficial effect of the preparation for the host. Some studies are pursuing this concept and providing evidence in favor of multi-species probiotics [83], but to our knowledge, there are no placebo-controlled head-to-head trials on the efficacy of single- versus multi-strain preparations in IBS.

There is large diversity between trials (Figure 3); most of the multi-strain preparations were nutritional supplements from various suppliers worldwide and the same product may be available in several countries under different brand names, with different labels and approved for different indications. 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 likely to be protected by patents. The use of *Streptococcus thermophilus* and *L. 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 as they were not regarded as probiotics until 2014, when the ISAPP consented to label them as probiotics by definition [67].

Other aspects of these studies also differ substantially; the number of participants ranged between 24 and 186, and treatment duration between 7 days to 6 months. Because of the lack of a common reporting period, (for example after 4 or 8 weeks), it is difficult to compare the outcomes of different studies. Not all studies reported the dosage of the microorganisms in the preparations, and based on the available data, doses differed by a factor of almost 1,000.

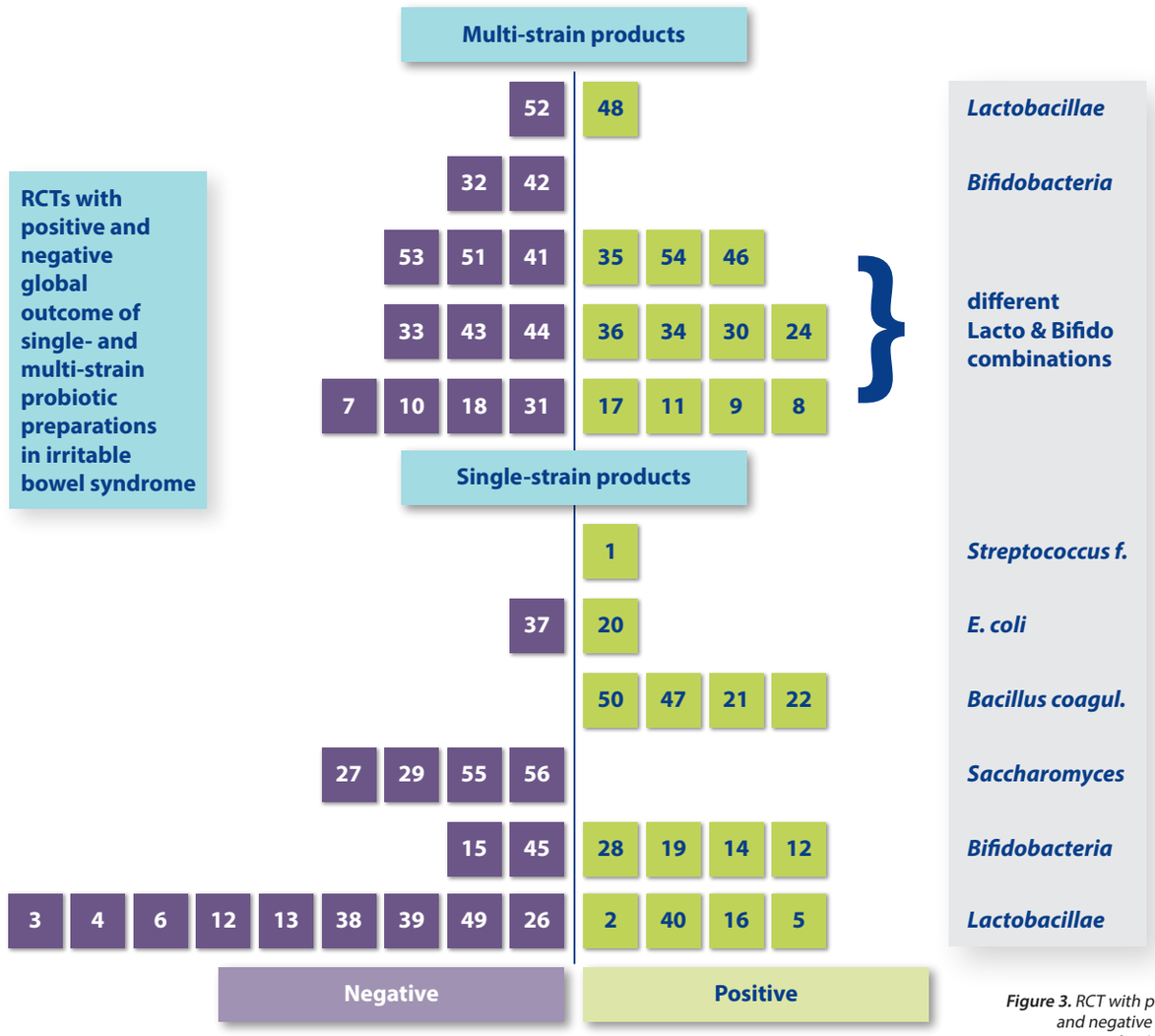


Figure 3. *RCT with positive and negative global outcome for different bacterial strains in multi-strain and single-strain probiotic RCT in IBS. Numbers refer to the numbers in the reference list.*

In summary we found that 14 of the 27 studies that used multi-strain preparations reported a negative outcome on overall symptoms; however, the treatment occasionally brought relief to some of the symptoms such as bloating and satiety. Quality of life improved in one study but symptomatic improvement was not different from placebo. Thirteen studies reported a positive effect on overall symptoms. Although the size of the populations used in the various studies has increased over the years, it had no apparent effect on outcomes, in fact, more patients were included in the studies reporting negative outcomes than in the studies with positive outcomes. The ratio of studies reporting positive and negative outcomes indicates that results are rather arbitrary and random and it remains inconclusive whether or not treatments of IBS using multi-strain probiotics are effective.

SINGLE-STRAIN PREPARATIONS

Studies using single-strain probiotics are less challenging than those using the multi-strain ones when attempting to demonstrate efficacy of probiotics in the treatment of IBS because they are comparable with traditional drug studies. We found 29 papers published between 1989 and 2014 that employed single-strain probiotics to modify IBS symptoms (Figure 3).

There is a large variability across studies with respect to design features; although most studies used a parallel group design, four of them were conducted using a cross-over design in which patients were included both in treatment and control arms consecutively. This is frequently used for motivational purposes, since patients are easier to recruit when they are offered effective treatment, at least for one period of the trial. However, we cannot assume that the data in both periods are equivalent and can be compared, as they may be biased by un-blinding and conditioning effects [84].

Studies also differ with respect to the treatment duration, which ranged from 4 to 12 weeks, and the number of participants, which ranged from n=12 to n=362. A small population sample leads to a low statistical power, poor reliability of the data, and inconclusive evidence. Moreover, the amount of living organisms in the preparations varied by a factor of almost 1,000, ranging from $1x10^8$ [14] to $8 x 10^{11}$ [27].

The most striking differences could be seen when the different species used to reduce IBS symptoms were analyzed separately. Six studies used *L. plantagus* of different origin and subspecies [3, 5, 6, 26, 40, 49] and since recent studies suggests that probiotic effects are strain-specific, it may have significant implications, i.e. strains belonging to the same species may or may not have different effects on IBS symptoms [85]. Other lactobacillus strains were only used in single trials, such as *L. brevi* [39], *L. acidophilus* [2, 16], *L. reuteri* [13], *L. rhamnosus GG* [4], and *L. casei* (CLR35) [38]. A similar picture emerges with bifidobacteria; individual trials have used *Bifidobacterium bifidum (MIMBb75)* [28], *B. lactis* [19], *B. animalis* [15] and *B. infantis* [14, 45]. Four studies investigated the effect of the yeast Saccharomyces on IBS, three used the *Saccharomyces boulardi* species [27, 29, 55] and the other one the *S. cerevisiae* species [56] but as is the case with lactic acid bacteria, it is not possible to recognize if they belong to the same strain and thus possess similar qualities. Only one single study used the *S. faecium* strain [1] and two used different *E. coli* strains, *E. coli Nissle* [37], and *E. coli* [20].

Collating the conclusions from all studies reveals that 16 of the 29 studies found negative or at least partly negative outcomes for the use of probiotics in the management of IBS. Pooling all patients from all studies, regardless of their respective findings, the number of patients who reported benefiting from probiotics was higher than the number of patients who did not. One study which compared a lactobacillus versus a bifidobacterium strain, both against placebo,[12] revealed that bifidobacterium was effective in relieving IBS symptoms while lactobacillus was not.

Overall, lactobacillus strains do not appear to be effective as nine out of 13 studies reported negative outcomes. Conversely, bifidobacteria seem to be effective in reducing IBS symptoms, with four out of six studies reporting positive results. All four studies using *Bacillus coagulans* had positive outcomes, although the size of their study population was small. One or two studies reported mixed results that were inconclusive and will require independent confirmation whilst all others reported negative results altogether (Saccharomyces).

CLINICALLY RELEVANT QUESTIONS AND ANSWERS

In the following we will discuss some more general questions that may be raised by practicing physicians in primary and secondary care.

Q1: Do probiotics help patients with IBS and what effect can I expect from a treatment?

As the pathophysiology of IBS remains unknown to a large extend, there is no opportunity for a "causal" treatment. Hence, the management of symptoms and improvement of patients' quality of life remain the main therapeutic goals. All previous meta-analyses, as well as our own data, suggest that some probiotics could reduce some symptoms of IBS. Probiotics could successfully influence bloating and abdominal pain [10, 19, 41, 56] but have a lesser impact on stool frequency and consistency in patients with constipation-predominant IBS type, with some studies showing positive [15] and others showing negative results [19]. The other essential outcome of treatment is improved quality of life [15, 27, 55] even when bowel symptoms are not significantly influenced.

Q2: Which probiotic should I prescribe, at which dosage, and how long should the treatment last?

Although the choice of a particular probiotic and treatment protocol is based on clinical efficacy, surveillance and colonisation abilities of specific strains should also be taken into consideration [86]. While it is not possible to draw a definite

conclusion with respect to any of the probiotic multi-strain preparations, single-strain agents containing bifidobacteria or *B. coagulans* have shown efficiency in patients with IBS, and so did some strains of *E. coli*. Moreover, bifidobacteria are shown to have specific "innate" mechanisms to resist in the aggressive environment of the human GI tract [87]. While most probiotic strains disappear from the bowel immediately after the termination of the treatment, some strains of *E. coli* were found up to 28 weeks after intake and are therefore good candidates for long lasting changes [88].

Probiotics that are distributed as drugs, as opposed to nutritional supplements, have their recommended dosages and these should not be exceeded during treatment. Usually, nutritional supplements should not contain less than 1 x 10^9 microorganisms in order to have some beneficial effect. Independently of the type of agent, treatment should last for at least 4 weeks to allow improvement in quality of life [89], unless clinical complications necessitate premature termination of treatment.

Q3: Where and when do probiotics have their role in the management of IBS?

Unlike other drug therapies, while there are number of national and international guidelines for the management of IBS, none provide an algorithm that can be used to determine what is the best protocol to follow for the clinical management of IBS [90]. Based on their averaged number needed to treat, probiotics rank very high when compared with other treatment options [91]. From our recent analyses we suggest that probiotics could well become the first therapeutic choice for IBS, even before conventional drugs such as spasmolytic agents, because of their comparatively low side-effect profile.

Two of three studies in our analysis that included constipation-predominant IBS patients [15, 19, 42] showed improvement in stool frequency and consistency, although the evidence for this group of patients was weak [89]. Both of them were using bifidobacteria as a treatment agent. In the diarrhea-predominant IBS subgroup, probiotics did not show promising results regarding stool consistency with only three studies (two using *B. coagulans*) reporting some improvement of symptoms.
However, abdominal pain, the predominant IBS complaint from patients, was improved in some studies [14, 18, 23, 28]. On the other hand, the improvement of patients' quality of life was evident in almost all studies reviewed. Irrespective of the mechanisms underlying this change, such as responses to doctors' attitudes and attention, this could be one of the most prominent indications for the prescription of probiotics in patients with IBS.

Q4: Are probiotics safe?

Treating chronic disorders, including IBS, can take several months or even several years; therefore it is crucial to ensure that medication will not induce long-term complications. Most probiotic studies reported no adverse events and amongst the very few which did, most adverse events were comparable with those observed in the control groups and where not believed to be drug related. Findings from studies investigating the use of probiotics in children with functional bowel pain or IBS also provide evidence supporting the long-term safety of probiotic treatments. In addition, some trials did not document any adverse events even though the dose of probiotic administered exceed over 1,000 times the recommended dosage [88].

CONCLUSIONS

In summary, we believe that the therapeutic use of probiotics for the treatment of patients with IBS should be individualized and tolerable for patients over a long period of time, as a minimum course of 4 weeks is required in order to be clinically meaningful. Secondly, probiotic species/strains that were shown to have an impact on overall IBS symptoms were bifidobacteria, *B. coagulans,* and *E. coli,* and these should be the preferred choice for treatment. Finally, pharmaceutical preparations with specific encapsulation of bacteria may offer a better chance of survival and enhance probiotic effects as opposed to fermented milk-based products. Future studies of probiotics, which follow FDA and EMA guidelines and include clinically relevant strains, will generate more reliable data. Stronger evidence will then be available to inform doctors in their everyday practice and patients, as already seen with other GI drugs.

References

(1) Gade J, Thorn P. Paraghurt for patients with irritable bowel syndrome: a controlled clinical investigation from general practice. Scand J Prim Health Care. 1989;7(1):23-6.

(2) Halpern GM, Prindiville T, Blankenburg M, Hsia T, Gershwin ME. Treatment of irritable bowel syndrome with Lacteol Fort: a randomized, double-blind, cross-over trial. Am J Gastroenterol. 1996;91(8):1579-85.

(3) Nobaek S, Johansson ML, Molin G, Ahrne S, Jeppsson B. Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. Am J Gastroenterol. 2000;95(5):1231-8.

(4) O'Sullivan MA, O'Morain CA. Bacterial supplementation in the irritable bowel syndrome: a randomised double-blind placebo-controlled crossover study. Dig Liver Dis. 2000;32(4):294-301.

(5) Niedzielin K, Kordecki H, Birkenfeld B. A controlled, double-blind, randomized study on the efficacy of Lactobacillus plantarum 299V in patients with irritable bowel syndrome. Eur J Gastroenterol Hepatol. 2001;13(10):1143-7.

(6) Sen S, Mullan MM, Parker TJ, Woolner JT, Tarry SA, Hunter JO. Effect of Lactobacillus plantarum 299v on colonic fermentation and symptoms of irritable bowel syndrome. Dig Dis Sci. 2002;47(11):2615-20.

(7) Kim HJ, Camilleri M, McKinzie S, Lempke MB, Burton DD, Thomforde GM, et al. A randomized controlled trial of a probiotic, VSL#3, on gut transit and symptoms in diarrhoea-predominant irritable bowel syndrome. Aliment Pharmacol Ther. 2003;17(7):895-904.

(8) Saggioro A. Probiotics in the treatment of irritable bowel syndrome. J Clin Gastroenterol. 2004;38(6 Suppl):S104-6.

(9) Bittner AC, Croffut RM, Stranahan MC. Prescript-Assist probiotic-prebiotic treatment for irritable bowel syndrome: a methodologically oriented, 2-week, randomized, placebo-controlled, double-blind clinical study. Clin Ther. 2005;27(6):755-61.

(10) Kim HJ, Vazquez Roque MI, Camilleri M, Stephens D, Burton DD, Baxter K, et al. A randomized controlled trial of a probiotic combination VSL# 3 and placebo in irritable bowel syndrome with bloating. Neurogastroenterol Motil. 2005;17(5):687-96.

(11) Kajander K, Hatakka K, Poussa T, Farkkila M, Korpela R. A probiotic mixture alleviates symptoms in irritable bowel syndrome patients: a controlled 6-month intervention. Aliment Pharmacol Ther. 2005;22(5):387-94.

(12) O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, et al. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology. 2005;128(3):541-51.

(13) Niv E, Naftali T, Hallak R, Vaisman N. The efficacy of Lactobacillus reuteri ATCC 55730 in the treatment of patients with irritable bowel syndrome--a double blind, placebo-controlled, randomized study. Clin Nutr. 2005;24(6):925-31.

(14) Whorwell PJ, Altringer L, Morel J, Bond Y, Charbonneau D, O'Mahony L, et al. Efficacy of an encapsulated probiotic Bifidobacterium infantis 35624 in women with irritable bowel syndrome. Am J Gastroenterol. 2006;101(7):1581-90.

(15) Guyonnet D, Chassany O, Ducrotte P, Picard C, Mouret M, Mercier CH, et al. Effect of a fermented milk containing Bifidobacterium animalis DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomized, double-blind, controlled trial. Aliment Pharmacol Ther. 2007;26(3):475-86.

(16) Sinn DH, Song JH, Kim HJ, Lee JH, Son HJ, Chang DK, et al. Therapeutic effect of Lactobacillus acidophilus-SDC 2012, 2013 in patients with irritable bowel syndrome. Dig Dis Sci. 2008;53(10):2714-8.

(17) Kajander K, Myllyluoma E, Rajilic-Stojanovic M, Kyronpalo S, Rasmussen M, Jarvenpaa S, et al. Clinical trial: multispecies probiotic supplementation alleviates the symptoms of irritable bowel syndrome and stabilizes intestinal microbiota. Aliment Pharmacol Ther. 2008;27(1):48-57.

(18) Drouault-Holowacz S, Bieuvelet S, Burckel A, Cazaubiel M, Dray X, Marteau P. A double blind randomized controlled trial of a probiotic combination in 100 patients with irritable bowel syndrome. Gastroenterol Clin Biol. 2008;32(2):147-52.

(19) Agrawal A, Houghton LA, Morris J, Reilly B, Guyonnet D, Goupil Feuillerat N, et al. Clinical trial: the effects of a fermented milk product containing Bifidobacterium lactis DN-173 010 on abdominal distension and gastrointestinal transit in irritable bowel syndrome with constipation. Aliment Pharmacol Ther. 2009;29(1):104-14.

(20) Enck P, Zimmermann K, Menke G, Klosterhalfen S. Randomized controlled treatment trial of irritable bowel syndrome with a probiotic E.-coli preparation (DSM17252) compared to placebo. Z Gastroenterol. 2009;47(2):209-14.

(21) Dolin BJ. Effects of a proprietary Bacillus coagulans preparation on symptoms of diarrhea-predominant irritable bowel syndrome. Methods Find Exp Clin Pharmacol. 2009;31(10):655-9.

(22) Hun L. Bacillus coagulans significantly improved abdominal pain and bloating in patients with IBS. Postgrad Med. 2009;121(2):119-24.

(23) Hong KS, Kang HW, Im JP, Ji GE, Kim SG, Jung HC, et al. Effect of probiotics on symptoms in Korean adults with irritable bowel syndrome. Gut Liver. 2009;3(2):101-7.

(24) Williams EA, Stimpson J, Wang D, Plummer S, Garaiova I, Barker ME, et al. Clinical trial: a multistrain probiotic preparation significantly reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled study. Aliment Pharmacol Ther. 2009;29(1):97-103.

(25) Simren M, Ohman L, Olsson J, Svensson U, Ohlson K, Posserud I, et al. Clinical trial: the effects of a fermented milk containing three probiotic bacteria in patients with irritable bowel syndrome - a randomized, double-blind, controlled study. Aliment Pharmacol Ther. 2010;31(2):218-27.

(26) Ligaarden SC, Axelsson L, Naterstad K, Lydersen S, Farup PG. A candidate probiotic with unfavourable effects in subjects with irritable bowel syndrome: a randomised controlled trial. BMC Gastroenterol. 2010;10:16.

(27) Choi CH, Jo SY, Park HJ, Chang SK, Byeon JS, Myung SJ. A randomized, double-blind, placebo-controlled multicenter trial of Saccharomyces boulardii in irritable bowel syndrome: effect on quality of life. J Clin Gastroenterol. 2011;45(8):679-83.

(28) Guglielmetti S, Mora D, Gschwender M, Popp K. Randomised clinical trial: Bifidobacterium bifidum MIMBb75 significantly alleviates irritable bowel syndrome and improves quality of life--a double-blind, placebo-controlled study. Aliment Pharmacol Ther. 2011;33(10):1123-32.

(29) Kabir MA, Ishaque SM, Ali MS, Mahmuduzzaman M, Hasan M. Role of Saccharomyces boulardii in diarrhea predominant irritable bowel syndrome. Mymensingh Med J. 2011;20(3):397-401.

(30) Ringel-Kulka T, Palsson OS, Maier D, Carroll I, Galanko JA, Leyer G, et al. Probiotic bacteria Lactobacillus acidophilus NCFM and Bifidobacterium lactis Bi-07 versus placebo for the symptoms of bloating in patients with functional bowel disorders: a double-blind study. J Clin Gastroenterol. 2011;45(6):518-25.

(31) Sondergaard B, Olsson J, Ohlson K, Svensson U, Bytzer P, Ekesbo R. Effects of probiotic fermented milk on symptoms and intestinal flora in patients with irritable bowel syndrome: a randomized, placebo-controlled trial. Scand J Gastroenterol. 2011;46(6):663-72.

(32) Hong YS, Hong KS, Park MH, Ahn YT, Lee JH, Huh CS, et al. Metabonomic understanding of probiotic effects in humans with irritable bowel syndrome. J Clin Gastroenterol. 2011;45(5):415-25.

(33) Michail S, Kenche H. Gut microbiota is not modified by randomized, double-blind, placebo-controlled trial of VSL#3 in diarrhea-predominant irritable bowel syndrome. Probiotics Antimicrob Proteins. 2011;3(1):1-7.

(34) Ki Cha B, Mun Jung S, Hwan Choi C, Song ID, Woong Lee H, Joon Kim H, et al. The effect of a multispecies probiotic mixture on the symptoms and fecal microbiota in diarrhea-dominant irritable bowel syndrome: a randomized, double-blind, placebo-controlled trial. J Clin Gastroenterol. 2012;46(3):220-7.

(35) Cui S, Hu Y. Multistrain probiotic preparation significantly reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled study. Int J Clin Exp Med. 2012;5(3):238-44.

(36) Min YW, Park SU, Jang YS, Kim YH, Rhee PL, Ko SH, et al. Effect of composite yogurt enriched with acacia fiber and Bifidobacterium lactis. World J Gastroenterol. 2012;18(33):4563-9.

(37) Kruis W, Chrubasik S, Boehm S, Stange C, Schulze J. A double-blind placebo-controlled trial to study therapeutic effects of probiotic Escherichia coli Nissle 1917 in subgroups of patients with irritable bowel syndrome. Int J Colorectal Dis. 2012;27(4):467-74.

(38) Dapoigny M, Piche T, Ducrotte P, Lunaud B, Cardot JM, Bernalier-Donadille A. Efficacy and safety profile of LCR35 complete freeze-dried culture in irritable bowel syndrome: a randomized, double-blind study. World J Gastroenterol. 2012;18(17):2067-75.

(39) Murakami K, Habukawa C, Nobuta Y, Moriguchi N, Takemura T. The effect of Lactobacillus brevis KB290 against irritable bowel syndrome: a placebo-controlled double-blind crossover trial. Biopsychosoc Med. 2012;6(1):16.

(40) Ducrotte P, Sawant P, Jayanthi V. Clinical trial: Lactobacillus plantarum 299v (DSM 9843) improves symptoms of irritable bowel syndrome. World J Gastroenterol. 2012;18(30):4012-8.

(41) Cappello C, Tremolaterra F, Pascariello A, Ciacci C, Iovino P. A randomised clinical trial (RCT) of a symbiotic mixture in patients with irritable bowel syndrome (IBS): effects on symptoms, colonic transit and quality of life. Int J Colorectal Dis. 2013;28(3):349-58.

(42) Roberts LM, McCahon D, Holder R, Wilson S, Hobbs FD. A randomised controlled trial of a probiotic 'functional food' in the management of irritable bowel syndrome. BMC Gastroenterol. 2013;13:45.

(43) Begtrup LM, de Muckadell OB, Kjeldsen J, Christensen RD, Jarbol DE. Long-term treatment with probiotics in primary care patients with irritable bowel syndrome--a randomised, double-blind, placebo controlled trial. Scand J Gastroenterol. 2013;48(10):1127-35.

(44) Ko SJ, Han G, Kim SK, Seo JG, Chung WS, Ryu B, et al. Effect of Korean herbal medicine combined with a probiotic mixture on diarrhea-dominant irritable bowel syndrome: a double-blind, randomized, placebo-controlled trial. Evid Based Complement Alternat Med. 2013;2013:824605.

(45) Charbonneau D, Gibb RD, Quigley EM. Fecal excretion of Bifidobacterium infantis 35624 and changes in fecal microbiota after eight weeks of oral supplementation with encapsulated probiotic. Gut Microbes. 2013;4(3):201-11.

(46) Yoon JS, Sohn W, Lee OY, Lee SP, Lee KN, Jun DW, et al. Effect of multispecies probiotics on irritable bowel syndrome: a randomized, double-blind, placebo-controlled trial. J Gastroenterol Hepatol. 2014;29(1):52-9.

(47) Urgesi R, Casale C, Pistelli R, Rapaccini GL, de Vitis I. A randomized double-blind placebo-controlled clinical trial on efficacy and safety of association of simethicone and Bacillus coagulans (Colinox®) in patients with irritable bowel syndrome. Eur Rev Med Pharmacol Sci. 2014;18(9):1344-53.

(48) Sisson G, Ayis S, Sherwood RA, Bjarnason I. Randomised clinical trial: a liquid multi-strain probiotic vs. placebo in the irritable bowel syndrome--a 12 week double-blind study. Aliment Pharmacol Ther. 2014;40(1):51-62.

(49) Stevenson C, Blaauw R, Fredericks E, Visser J, Roux S. Randomized clinical trial: effect of Lactobacillus plantarum 299 v on symptoms of irritable bowel syndrome. Nutrition. 2014;30(10):1151-7.

(50) Rogha M, Esfahani MZ, Zargarzadeh AH. The efficacy of a synbiotic containing Bacillus coagulans in treatment of irritable bowel syndrome: a randomized placebo-controlled trial. Gastroenterol Hepatol Bed Bench. 2014;7(3):156-63.

(51) Shavakhi A, Minakari M, Farzamnia S, Peykar MS, Taghipour G, Tayebi A, et al. The effects of multi-strain probiotic compound on symptoms and quality-of-life in patients with irritable bowel syndrome: a randomized placebo-controlled trial. Adv Biomed Res. 2014;3:140.

(52) Lorenzo-Zuniga V, Llop E, Suarez C, Alvarez B, Abreu L, Espadaler J, et al. I.31, a new combination of probiotics, improves irritable bowel syndrome-related quality of life. World J Gastroenterol. 2014;20(26):8709-16.

(53) Ludidi S, Jonkers DM, Koning CJ, Kruimel JW, Mulder L, van der Vaart IB, et al. Randomized clinical trial on the effect of a multispecies probiotic on visceroperception in hypersensitive IBS patients. Neurogastroenterol Motil. 2014;26(5):705-14.

(54) Jafari E, Vahedi H, Merat S, Momtahen S, Riahi A. Therapeutic effects, tolerability and safety of a multi-strain probiotic in Iranian adults with irritable bowel syndrome and bloating. Arch Iran Med. 2014;17(7):466-70.

(55) Abbas Z, Yakoob J, Jafri W, Ahmad Z, Azam Z, Usman MW, et al. Cytokine and clinical response to Saccharomyces boulardii therapy in diarrhea-dominant irritable bowel syndrome: a randomized trial. Eur J Gastroenterol Hepatol. 2014;26(6):630-9.

(56) Pineton de Chambrun G, Neut C, Chau A, Cazaubiel M, Pelerin F, Justen P, et al. A randomized clinical trial of Saccharomyces cerevisiae versus placebo in the irritable bowel syndrome. Dig Liver Dis. 2015;47(2):119-24.

(57) McFarland LV, Dublin S. Meta-analysis of probiotics for the treatment of irritable bowel syndrome. World J Gastroenterol. 2008;14(17):2650-61.

(58) Nikfar S, Rahimi R, Rahimi F, Derakhshani S, Abdollahi M. Efficacy of probiotics in irritable bowel syndrome: a meta-analysis of randomized, controlled trials. Dis Colon Rectum. 2008;51(12):1775-80.

(59) Brenner DM, Moeller MJ, Chey WD, Schoenfeld PS. The utility of probiotics in the treatment of irritable bowel syndrome: a systematic review. Am J Gastroenterol. 2009;104(4):1033-49.

(60) Hoveyda N, Heneghan C, Mahtani KR, Perera R, Roberts N, Glasziou P. A systematic review and meta-analysis: probiotics in the treatment of irritable bowel syndrome. BMC Gastroenterol. 2009;9:15.

(61) Moayyedi P, Ford AC, Talley NJ, Cremonini F, Foxx-Orenstein AE, Brandt LJ, et al. The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. Gut. 2010;59(3):325-32.

(62) Whelan K. Probiotics and prebiotics in the management of irritable bowel syndrome: a review of recent clinical trials and systematic reviews. Curr Opin Clin Nutr Metab Care. 2011;14(6):581-7.

(63) Ritchie ML, Romanuk TN. A meta-analysis of probiotic efficacy for gastrointestinal diseases. PLoS One. 2012;7(4):e34938.

(64) Ford AC, Quigley EM, Lacy BE, Lembo AJ, Saito YA, Schiller LR, et al. Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. Am J Gastroenterol. 2014;109(10):1547-61.

(65) Didari T, Mozaffari S, Nikfar S, Abdollahi M. Effectiveness of probiotics in irritable bowel syndrome: updated systematic review with meta-analysis. World J Gastroenterol. 2015;21(10):3072-84.

(66) Mazurak N, Broelz E, Storr M, Enck P. Probiotic therapy of the irritable bowel syndrome: why is the evidence still poor and what can be done about It? J Neurogastroenterol Motil. 2015;21(4):471-85.

(67) Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11(8):506-14.

(68) Food and Agriculture Organisation, World Health Organisation. Evaluation of health and nutritional properties of probiotics in food, including powder milk with live lactic acid bacteria. FAO & WHO Expert Consultation Report, Geneva, 2001.

(69) Thompson WG. The road to Rome. Gastroenterology. 2006;130(5):1552-6.

(70) Tack J, Fried M, Houghton LA, Spicak J, Fisher G. Systematic review: the efficacy of treatments for irritable bowel syndrome--a European perspective. Alimentary Pharmacology and Therapy. 2006;24(2):183-205.

(71) Surawicz CM, Elmer GW, Speelman P, McFarland LV, Chinn J, van Belle G. Prevention of antibiotic-associated diarrhea by Saccharomyces boulardii: a prospective study. Gastroenterology. 1989;96(4):981-8.

(72) Oksanen PJ, Salminen S, Saxelin M, Hamalainen P, Ihantola-Vormisto A, Muurasniemi-Isoviita L, et al. Prevention of travellers' diarrhoea by Lactobacillus GG. Ann Med. 1990;22(1):53-6.

(73) Isolauri E, Kaila M, Mykkanen H, Ling WH, Salminen S. Oral bacteriotherapy for viral gastroenteritis. Dig Dis Sci. 1994;39(12):2595-600.

(74) McFarland LV, Surawicz CM, Greenberg RN, Fekety R, Elmer GW, Moyer KA, et al. A randomized placebo-controlled trial of Saccharomyces boulardii in combination with standard antibiotics for Clostridium difficile disease. JAMA. 1994;271(24):1913-8.

(75) Isolauri E, Juntunen M, Rautanen T, Sillanaukee P, Koivula T. A human Lactobacillus strain (Lactobacillus casei sp strain GG) promotes recovery from acute diarrhea in children. Pediatrics. 1991;88(1):90-7.

(76) Kim SE, Choi SC, Park KS, Park MI, Shin JE, Lee TH, et al. Change of fecal flora and effectiveness of the short-term VSL#3 probiotic treatment in patients with functional constipation. J Neurogastroenterol Motil. 2015;21(1):111-20.

(77) Kruis W, Schutz E, Fric P, Fixa B, Judmaier G, Stolte M. Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis. Aliment Pharmacol Ther. 1997;11(5):853-8.

(78) Chey WD, Kurlander J, Eswaran S. Irritable bowel syndrome: a clinical review. JAMA. 2015;313(9):949-58.

(79) Enck P, Aziz Q, Barbara G, Farmer AD, Fukudo S, Mayer E, et al. Irritable bowel syndrome: a clinical review. Nat Rev Dis Primers. (in press).

(80) Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci. 2012;13(10):701-12.

(81) Farmer AD, Randall HA, Aziz Q. It's a gut feeling: how the gut microbiota affects the state of mind. J Physiol. 2014;592(Pt 14):2981-8.

(82) Higgins JPT, Green S. Cochrane handbook for systematic reviews of interventions: Version 5.1.0 [updated March 2011]. Available from URL: www.cochrane-handbook.org.

(83) Zoppi G, Cinquetti M, Benini A, Bonamini E, Minelli EB. Modulation of the intestinal ecosystem by probiotics and lactulose in children during treatment with ceftriaxone. Curr Ther Res Clin Exp. 2001;62(5):418-35.

(84) Weimer K, Enck P. Traditional and innovative experimental and clinical trial designs and their advantages and pitfalls. Handb Exp Pharmacol. 2014;225:237-72.

(85) Food and Agriculture Organisation, World Health Organisation. Probiotics in food: health and nutritional properties and guidelines for evaluation - FAO Food and Nutrition Paper 85. 2006.

(86) Prilassnig M, Wenisch C, Daxboeck F, Feierl G. Are probiotics detectable in human feces after oral uptake by healthy volunteers? Wiener Klinische Wochenschrift. 2007;119(15-16):456-62.

(87) Gonzalez-Rodriguez I, Ruiz L, Gueimonde M, Margolles A, Sanchez B. Factors involved in the colonization and survival of bifidobacteria in the gastrointestinal tract. Fems Microbiology Letters. 2013;340(1):1-10.

(88) Wassenaar TM, Beimfohr C, Geske T, Zimmermann K. Voluntarily exposure to a single, high dose of probiotic Escherichia coli results in prolonged colonisation. Beneficial Microbes. 2014;5(4):367-75.

(89) Hungin AP, Mulligan C, Pot B, Whorwell P, Agreus L, Fracasso P, et al. Systematic review: probiotics in the management of lower gastrointestinal symptoms in clinical practice -- an evidence-based international guide. Aliment Pharmacol Ther. 2013;38(8):864-86.

(90) Rao VL, Cifu AS, Yang LW. Pharmacologic management of irritable bowel syndrome. JAMA. 2015;314(24):2684-5.

(91) Enck P, Junne F, Klosterhalfen S, Zipfel S, Martens U. Therapy options in irritable bowel syndrome. Eur J Gastroenterol Hepatol. 2010;22(12):1402-11.

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FAECALIBACTERIUM PRAUSNITZII A2-165 HAS A HIGH CAPACITY TO INDUCE IL-10 IN HUMAN AND MURINE DENDRITIC CELLS AND MODULATES T CELL RESPONSES

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Faecalibacterium prausnitzii strain A2-165 was already known for its anti-inflammatory properties and has been shown to prevent colitis in a 2,4,6-trinitrobenzenesulfonic acid model. Previous studies have found that patients with Crohn's disease and ulcerative colitis have lower fecal counts of *F. prausnitzii* and increased abundance of Proteobacteria. The Proteobacteria phylum includes the major pathobiont *Escherichia coli,* which contributes to the disease pathogenesis. The enteric immune system has dendritic cells and T cells, which are the major players of inflammation in patients with inflammatory bowel disease (IBD). Although numerous genetic mutations in patients with IBD have been discovered, the exact mechanism of action of these genes in IBD is still unknown.

Researchers from the Netherlands, the United Kingdom, and France investigated the effect of *F. prausnitzii* on the cells of the immune system and their cytokine secretions. The immunomodulatory properties of strain A2-165 were compared in vitro with four different *F. prausnitzii* isolates and eight abundant intestinal commensals using human dendritic cells and mouse bone marrow-derived dendritic cells (BMDCs). Their analysis showed that cytokine responses to *F. prausnitzii* A2-165 were particularly different to others, as it induced a high amount of interleukin (IL)-10 in dendritic cells. In order to confirm these findings *in vivo*, a mouse dinitrobenzenesulfonic acid (DNBS) model, which simulates a relapsing type of IBD, was used. F. prausnitzii A2-165, a high secretor of IL-10, and *Clostridium hathewayi,* a low secretor of IL-10, were given to the animals and the Th1-driven inflammatory responses to DNBS were investigated. They showed that only the *F. prausnitzii* strain A2-165 triggered an anti-inflammatory response. A mouse model of nasal tolerance to ovalbumin showed an increased ovalbumin-specific T cell proliferation to *F. prausnitzii* A2-165. Similarly, in vitro BMDCs were stimulated by F. prausnitzii A2-165 to induce the proliferation of ovalbumin-specific T cells and the decrease of interferon (IFN)-γ (+) T cells.

In conclusion, *F. prausnitzii* A2-165 has the potential to induce dendritic cells to secrete IL-10 and promote the differentiation of T cells *in vitro* and *in vivo*. Reduction of IFN-γ secretions also contributes to the anti-inflammatory, colitis-suppressive effect of *F. prausnitzii* in mouse colitis models. This study shows the potential for *F. prausnitzii* A2-165 to act as a probiotic in patients with IBD. Finding therapies for IBD and gut microbiota is currently a hot topic of research and tremendous progress has already been made over the last decade. Therapeutic strategies using microbiota modulation is a new frontier and *F. prausnitzii* might be a potential candidate for microbiota modulation in patients with IBD.

ORAL VERSUS INTRAVENOUS IRON REPLACEMENT THERAPY DISTINCTLY ALTERS THE GUT MICROBIOTA AND METABOLOME IN PATIENTS WITH IBD

Lee T, Clavel T, Smirnov K, Schmidt A, Lagkouvardos I, Walker A, et al. Gut. 2016 Feb 4 [E-pub ahead of print]. doi: 10.1136/gutjnl-2015-309940.

Iron deficiency anemia is a debilitating condition in IBD and adjustment of iron levels leads to improved quality of life. Although, parenteral iron replacement therapy (IRT) is considered a safe and practical approach, there are conflicting reports about the route of IRT and the effects on patients. IBD is a complex disease with many etiological factors. Recent advances in genetic microbiology have shown a strong association between dysbiosis and the occurrence and progression of IBD. Oral iron supplementation has previously been found to influence the gut microbiota, affecting the dominant phylum of bacteria and sometimes causing dysbiosis. However, there are no comparative studies highlighting the differences between oral and parenteral IRT on the gut microbiota composition.

In this study, the effects of oral versus parenteral IRT were examined in 31 patients with Crohn's disease (CD), 22 patients with ulcerative colitis (UC) and 19 control subjects (non-inflamed; NI). After randomization, participants received oral iron sulfate (PO) or intravenous iron sucrose (IV) over 3 months. Clinical activity index, gut microbiota profiles and metabolic parameters were recorded for all patients. A total of 72 participants completed the study (36 in each of the PO and IV group), including 19 NI patients, and 53 patients with IBD (31 with CD and 22 with UC). After intervention, the mean iron saturation level in the control group was no different between the PO and IV routes. The route of IRT did not affect disease activity based on changes in the clinical disease activity indices (modified Harvey-Bradshaw Index and Partial Mayo Scoring Index) and serum concentrations of C-reactive protein. There was a trend towards higher magnitude of improved Short Inflammatory Bowel Disease Questionnaire scores in the IBD IV-IRT groups, but results did not reach significance. Quality of life was improved in patients with CD in the IV-IRT cohort only.

Gut microbiota in patients with IBD were different to what had previously been reported. Interestingly, patients with CD in the IV-IRT cohort were reported to have the highest inter-individual differences as characterized by the composition of fecal bacterial communities after 3 months of treatment. In accordance with many previous trials, Clostridiales species were decreased in patients with CD; Clostridiales species are thought to play a protective role in CD by regulating T cell responses. The transition from a quiescent state to disease exacerbation in patients with IBD was shown to be associated with alterations of fecal bacterial communities, which supports the hypothesis that inflammatory processes in the intestines of both patients with UC and patients with CD affect the gut microbiota. However, in this study, researchers were unable to show an association between clinical flares and IV-IRT-related dysbiosis, possibly due to a relatively short follow-up period. In conclusion, although clinical endpoints of IV- and PO-IRT did not differ, PO-IRT induced dysbiosis in patients with IBD. Further large scale trials with longer follow-up periods are needed to confirm these findings.

References

Heida FH, van Zoonen AG, Hulscher JB, Te Kiefte BJ, Wessels R, Kooi EM, et al. A necrotizing enterocolitis-associated gut microbiota is present in the meconium: results of a prospective study. Clin Infect Dis. 2016 Jan 19 [E-pub ahead of print]. pii: ciw016.

Liu S, da Cunha AP, Rezende RM, Cialic R, Wei Z, Bry L, et al. The Host shapes the gut microbiota via fecal microRNA. Cell Host Microbe. 2016;19(1):32–43.

A NECROTIZING ENTEROCOLITIS-ASSOCIATED GUT MICROBIOTA IS PRESENT IN THE MECONIUM: RESULTS OF A PROSPECTIVE STUDY

Heida F, van Zoonen A, Hulscher J, te Kiefte B,Wessels R, Kooi E, et al. Clin Infect Dis. 2016 Jan 19 [E-pub ahead of print]. pii: ciw016.

Necrotizing enterocolitis (NEC) is a serious inflammatory bowel disease with a high mortality rate (20–30%) in preterm neonates. Although researchers have found that profound dysbiosis is associated with NEC, the timing and pathogenesis of this association is not thoroughly understood. Previous studies indicated a decreased diversity and increased pathobiont colonization (such as Clostridiales species, *Klebsiella pneumoniae,* and *Escherichia coli*) in patients with NEC. Although the association of these gut microbiota alterations is clear, the gap between birth and time to NEC development remains a mystery.

Heida and colleagues, investigated the gut microbiota profile of preterm infants from birth to the development of NEC. A case-control study with a cohort of neonates at high risk for NEC (n=11), coded as NTR4153 in the Dutch trial registry, and control subjects (n=22) was performed. Every patient with NEC was matched with two control subjects for gestational age and body weight, according to the availability of samples from patients of the same postnatal age as their matched counterpart. Fecal samples were collected twice a week from birth until the development of NEC, DNA was extracted, and the bacterial 16S rRNA genes were analyzed on a MiSeq sequencer.

The first fecal samples were collected at a median of 1 day (range 0–4) after birth. All of these samples consisted of meconium. The last two samples prior to NEC development were collected at a median of 5 days (range 2–7) and 2 days (range 0–4), respectively. In the meconium samples, both *Clostridium perfringens* and *Bacteroides dorei* were significantly higher in patients who developed NEC compared with those who did not ($p<0.001$), whereas the abundance of *C. difficile* was lower in meconium samples from patients who developed NEC. Contrary to previous studies, bacterial diversity did not differ between groups, possibly because the amount of pathobiont species contributed to increased diversity in NEC patients. Breast-feeding was a protective factor just before the onset of NEC.

This study demonstrates the presence of a NEC-associated gut microbiota present in the meconium, that contains *C. perfringens* and *B. dorei*. Therefore, this study suggests that factors during the first days of life, during delivery or even in utero, might affect the formation of a NEC-associated microbiota. It is interesting to note that both meconium and fetus were considered sterile a decade ago. However, it seems that bacterial colonization starts earlier than previously thought and recent reports suggest that in utero microbiota is associated with preterm delivery. This study further supports the hypothesis that not only meconium but also the microbiota profile in utero could play an important role in the development of NEC. This is the first study to show that the characteristics of the meconium and the changes in the composition of the gut microbiota in the weeks following birth are critical to the development of NEC.

This study has some limitations, including the lack of microbiota profiles for the amniotic fluid, maternal stool, and breast milk. Further studies that focus on analyzing the microbiome together with other confounding factors will provide valuable data and further our understanding with regard to characteristics that can be used to predict and prevent NEC and the available therapies for patients who develop this potentially deadly condition.

THE HOST SHAPES THE GUT MICROBIOTA VIA FECAL MICRO RNA

Liu S, Pires da Cunha A, Rezende R, Cialic R, Wei Z, Bry L, et al. Cell Host Microbe 2016;19(1):32–43.

In humans, the gut microbiota is often considered the second genome and a virtual organ of the gastrointestinal (GI) system. The development of highly sophisticated molecular genetic analysis systems has progressively led to the realization that the gut microbiota is not only an organ of the GI system but that much more complex interactions exist between the immune system, neuropsychiatric conditions, carcinogenesis, and so on. According to the Human Microbiome Project, the gut microbiota is relatively stable from birth to death, and almost all human beings possess a core gut microbiome. Many factors affecting the gut microbiota have been determined, including diet, gastric acidity, antibiotics, proton pump inhibitors, host genetics, and the brain-gut axis. However, the exact pathogenesis of this cross-talk is still unknown. From studies involving reciprocal transplantations of gut microbiotas into different hosts, it is known that the recipient's microenvironment influences the composition of the transplanted microbiota so that it resembles that of the host. This suggests that the host has the capability to selectively keep some of the species of microorganisms in their gut environment.

MicroRNAs (miRNAs) are small non-coding RNAs, which selectively modify post-transcriptional regulation of gene expression. They have been shown to act as disease-modifiers in many conditions such as cancer, obesity, nervous system disorders, and inflammation. Although many studies have focused on serum levels of miRNAs in many conditions, there are very few data available on fecal levels of miRNAs.

Liu and colleagues have published a thorough analysis of how gut microbiota is shaped by the host. The study shows that fecal miRNA-mediated gene regulation affects recipients gut microbiota. miRNAs are abundant in mouse and human fecal samples and present within extracellular vesicles. The loss of the miRNA-processing enzyme Dicer in intestinal epithelial cells (IEC) and Hopx-positive cells is the main reason for the presence of fecal miRNA . These miRNAs enter bacterial cells and regulate their growth by changing the expression of certain genes related to bacterial growth. In IEC-miRNA-deficient (Dicer1DIEC) mice, the lack of regulation of gut bacterial growth results in inflammation; this dysbiosis can be reversed by transplanting fecal miRNA from wild-type mice, which attenuates colitis.

In summary, miRNAs in feces regulate gene expression in gut bacteria and influence their growth. It is likely, although not yet proven, that the same mechanism applies to fungi and viruses present in the gut. Even though these miRNAs are clearly sensitive to environmental factors such as diet and antibiotics, it is likely that the host genetics also affect them substantially. This year, another study showed that fucosylation patterns in patients with ulcerative colitis determine the gut microbiome. Fucosylation is a process implicated in the secretions of blood group antigens in the mucosa of the GI tract. These blood group receptors also serve as receptors for organisms of the gut microbiota to attach selectively. It is now understood that the human gut microbiome is mainly shaped by host epigenetic factors. A lot still need to be uncovered in order to identify the mechanisms underlying microbiome changes in specific diseases; however this study is another step forward and contributes to gaining a deeper understanding in this area. The modulation of the gut microbiota through epigenetics might be the next frontier in the treatment of many GI and non-GI diseases.

THE GUT MICROBIOTA IN THE UNITED GASTROENTEROLOGY WEEK 2015

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INTRODUCTION

This year's United Gastroenterology Week (UEGW) took place on October 24th to 28th in Barcelona. Studies of the gut microbiota are becoming a prevalent topic among the original submissions to the congress, and a total of 55 abstracts focusing on gut microbial communities were presented. An additional set of 14 original research articles dealt with the gastrointestinal (GI) effects of probiotic supplementation. This article will briefly summarize data and knowledge gained from some of these studies.

The luminal microbiota (LM) and the mucosal-associated microbiota (MAM) are two distinct ecosystems and are thought to have different metabolic and immunological functions. Characterization of bacterial communities was done using high throughput pyrosequencing of the 16S rRNA gene in fecal and colonic mucosal biopsy samples from 16 patients with irritable bowel syndrome (IBS) [1]; biopsies were obtained without previous bowel cleansing. As previously shown in healthy subjects, differences in abundance of dominant phyla were found between the LM and MAM niches: Firmicutes (41% vs 28%), Actinobacteria (20% vs 12%) and Proteobacteria (11% vs 20%). Interestingly, the LM showed higher species diversity and tighter clustering of species than the MAM niche, a finding that might suggest robustness of the luminal ecosystem. Genera belonging to the Proteobacteria and Bacteroidetes phyla were more abundant in the MAM than in the LM. This study highlights the need to consider these two microbial niches of the GI tract in order to better understand the role of the intestinal microbiota in health and disease.

Another study evaluated the differences in the composition of the gut microbiota between 15 patients with inflammatory bowel disease (IBD), three patients with IBS, four patients with diverticular disease (DD) and eight controls [2]. Compared with microbiotas from other subjects, the composition of the microbiota of patients with IBD differed significantly. Proteobacteria were increased in all diseased groups compared with the control group, while Actinobacteria were increased in IBD and DD groups. The most represented species in IBD and DD was *Collinsella aerofaciens,* whereas *Faecalibacterium prausnitzii* was under-represented in patients with IBD. The chao1 score for assessing species diversity was similar across control, IBS and DD groups, but was reduced in patients with IBD. The authors suggested that a continuous dysbiotic spectrum exists in these GI diseases and IBD displays the most extreme changes in the composition of the gut microbiota.

Julien Tap and colleagues [3] studied fecal and mucosal microbiota in 130 subjects, including 95 patients with IBS (ROME III, all subtypes, n=78 severe IBS) and 35 healthy controls. Symptoms in patients with IBS were thoroughly characterized for severity (IBS-SSS, GSRS). 16S rRNA microbiota operational taxonomic unit (OTU)-based data complexity was reduced using a machine learning procedure into a "species-specific IBS severe signature", consisting of 100 bacterial OTUs (extracted from a total of 2900 OTUs) linked to IBS severity as assessed by IBS-SSS. This IBS severity microbial signature was further confirmed in sigmoid mucosal microbiota (n=57, AUC=0.80) and with an external validation stool set (n=46, AUC=0.68), discriminating patients with severe IBS from patients with mild/moderate IBS and healthy controls. Using this OTU-based signature, IBS symptom severity score was significantly and negatively associated with 1) exhaled methane, 2) presence of Archaea methanogens 3) microbial species richness and 4) enterotypes enriched either with Clostridiales or Prevotella species.
In conclusion, the authors provide some evidence suggesting that symptom severity in IBS is associated with distinct signatures at the fecal microbiota level.

FECAL MICROBIOTA TRANSPLANTATION

In two research articles, Cammarota and colleagues assessed the effectiveness of fecal microbiota transplantation (FMT) in the treatment of recurrent *Clostridium difficile* infection (rCDI). The first presentation reported the clinical outcomes of a group of patients treated with FMT for rCDI in a tertiary care center over a period of 2 years [4]. Thirty-five subjects received FMT from healthy donors, eight patients received multiple infusions, and all procedures were performed by colonoscopy. Resolution of rCDI occurred in 33 of the 35 treated patients (94%). *Klebsiella pneumoniae*-related sepsis occurred in one patient, and two patients, with concomitant urinary infections, presented a transient, self-limiting bacteremia after FMT. Two subjects died because of overwhelming rCDI after failure of FMT. Eight patients died 6 to 12 months after FMT because of comorbidities, mainly cardiovascular disease, not relatable to the procedure. In a second study, the same investigators considered the need for surgery in rCDI patients after the implementation of the FMT program in the center [5]. This retrospective study showed that the FMT program was associated with a reduction of surgical procedures for rCDI

despite an increasing epidemic of the infection. Together, these studies highlight the importance of FMT in the management of rCDI.

Hale and colleagues [6] examined the gut microbial composition of 115 patients with non-*C. difficile* diarrhea and compared it with 92 patients with *C. difficile* infection (CDI) and 110 healthy volunteers. Among the patients with *C. difficile*-negative diarrhea, 16 had Crohn's disease (CD), 13 had ulcerative colitis (UC), seven had viral gastroenteritis, 21 had IBS, and 56 had other causes of diarrhea. Gut microbial communities from patients with CDI were significantly different from healthy volunteers; a subset of patients with *C. difficile*-negative diarrhea (n=54) had markedly altered gut microbial communities characterized by lower relative abundances of Bacteroidetes and higher relative abundances of Proteobacteria. These alterations were in accordance with gut microbial changes observed previously in patients with CDI and, interestingly, were associated with clinical risk factors commonly linked to the development of CDI, such as recent antibiotic use (odds ratio [OR]: 0.24; 95% confidence interval [CI] 0.08-0.67), immunosuppression (OR:0.32; 95% CI 0.13-0.77), current hospitalization (OR:0.21; 95% CI 0.06-0.68), recent hospitalization (OR:0.26; 95% CI 0.07-0.83), and prior CDI (OR:0.06; 95% CI 0.001-0.43). The remaining *C. difficile*-negative patients (n=61) had gut microbial communities that resembled those of healthy volunteers. Thus, patients with *C. difficile*-negative diarrhea but clinical risk factors associated with CDI exhibit gut microbial alterations similar to those seen in CDI. Would those common risk factors be responsible for the microbial changes observed in both subsets of patients?

Preliminary results of an open label feasibility trial on FMT in patients with mild-moderate UC were presented in poster format [5]. Scaldaferri and colleagues enrolled 15 patients with active UC (partial Mayo score ≥4 with an endoscopic Mayo ≥1 with no upper limit on Mayo score). Eight patients received three administrations of FMT using 200 CC of fecal slurry from a healthy donor proposed by the patient and 7 patients received standard therapy. Primary outcome was feasibility and safety of FMT. Secondary end points were: clinical remission (partial Mayo score ≤2 with no sub score ≥1), clinical response (reduction of Mayo score of at least 2 points at week 2, 6, 12) and endoscopic remission (Mayo score = 0 at week 6). From the FMT group, one serious adverse event (kidney stone) was reported, and two patients discontinued due to disease worsening, whilst in the standard therapy group, one serious adverse event (cerebral arterial thrombosis) and one infusion reaction were reported and two patients discontinued due to disease worsening. At week 2, clinical remission and clinical response rate was somehow better for standard therapy (14.3% and 57.1%, respectively) than for FMT (25% and 25%). However, at week 12, response rates were better for FMT (37.5% and 50%) than for standard therapy (28.6% and 28.6%). FMT appears to be safe and may induce persistent clinical responses, but further studies are mandatory to confirm these results.

Another study investigated the impact of antibiotic treatment before FMT for refractory chronic active UC [7]. Twenty-seven patients with chronic active UC were treated with antibiotic therapy for 10 days. Subsequently, 17 patients received FMT via colonoscopy into the right colon, which was repeated in 14 days intervals by sigmoidoscopy for a total of 5 applications. The other 10 patients received antibiotic triple therapy only. Results showed that antibiotic treatment led to an overall reduction of the Mayo score from 8.4 to 6.8 in all patients within 10 days. Moreover, FMT showed an additional benefit in the follow-up period of 30 weeks (total Mayo score in the FMT group from 9.0 to 4.7 points vs. 7.5 to 6.3 in the antibiotic triple therapy group). Adherence to therapy during follow up in the antibiotic triple therapy group was lower (5/10), due to *C. difficile* infection (3/10), acute UC flare (1/10) and antibiotic-associated diarrhea (1/10), while adherence in the FMT group was 100%. At day 90, four patients achieved clinical remission (total Mayo score ≤2), and 6 patients had a partial response (reduction of total Mayo score ≥3 points) in the FMT group, whereas 2 patients had a partial response in the antibiotic triple therapy group. Hence, antibiotic treatment before FMT may confer a clinical benefit in chronic active UC.

INFLAMMATORY BOWEL DISEASE

CERTIFI was a Phase 2b multicenter, randomized, double-blind, placebo controlled clinical trial that assessed the efficacy and safety of ustekinumab therapy in subjects with moderately to severely active CD who had previously not responded to anti-tumor necrosis factor (TNF) therapy [8]. In this study, fecal samples from 100 subjects, collected at screening and on week 4, week 6, and week 22, were analyzed. The gut microbiota was characterized by pronounced interpersonal variation both in presence and relative abundance of specific bacterial taxa. Baseline CD activity index (CDAI) score was significantly associated with the relative abundance of several bacteria, including Parabacteroides taxa. Baseline C-reactive protein, fecal calprotectin, and lactoferrin concentrations also correlated with baseline bacterial abundances of specific taxa. Previous response to anti-TNF therapy did not significantly correlate with the abundance of any specific bacteria.

Scaldaferri and colleagues [9] evaluated the gut microbiota composition in patients with CD before and after anti-TNF-α induction treatment. Reduction of Enterobacteriacae and Ruminococcus together with increase in Bacteroidetes and *F. prausnitzii* was associated with clinical response to anti-TNF-α.

Magnusson and colleagues determined antimicrobial peptides (AMP) and microbiota profiles in anti-TNF therapy-naïve patients with UC before treatment and compared this data with anti-TNF therapy outcomes [10]. Gene expression of 11 AMPs or genes associated with AMP expression were analyzed in biopsies. Multivariate data analysis showed that responders and non-responders clustered differently when studying mRNA levels of the 11 genes. The most important nominators for therapy response were increased expression of defensin 5 and eosinophil cationic protein, and decreased expression of cathelicidin. Microbiota analysis of fecal samples (four responders and three non-responders) revealed that non-responders tended to have higher dysbiosis indexes compared with responders (p=0.097). Also, non-responders had low levels of *F. prausnitzii* while responders showed normal levels. These results suggest that response to anti-TNF therapy may benefit from a defined antimicrobial defense pattern.

Two poster presentations explored the fungal composition of the fecal and mucosal microbiota in patients with IBD. Sokol and colleagues [11] studied the bacterial and fungal composition of the fecal microbiota of patients with IBD and healthy subjects. Among the 235 patients with IBD (106 in flare, 129 in remission), 149 patients had CD and 86 had UC. The results of the bacterial microbiota analysis were in accordance with published data. Beta diversity analysis showed that samples clustered according to disease activity both for bacterial and fungal microbiota. Fungal microbiota in both patients with IBD and healthy subjects was dominated by the Basidiomycota and Ascomycota phyla, and by the Saccharomyces, Debaryomyces, Penicillium, and Candida genera. The fungal gut microbiota was found imbalanced in patients with IBD, with an increase in the Basidiomycota:Ascomycota ratio and a decreased proportion of Saccharomyces and Kluyveromyces compared with healthy subjects. Fungal biodiversity was also decreased in patients with IBD, particularly in those with colon involvement. Authors concluded that the fungal microbiota is imbalanced in patients with IBD with a reduced biodiversity and an increased Basidiomycota:Ascomycota ratio compared with healthy subjects.

Another study by the same group examined the bacterial and fungal composition of MAM in 23 patients with CD (16 in flare and seven in remission) and in ten healthy subjects [12]. Overall, fungi load was significantly increased in patients with CD in flare compared with healthy subjects. Dioszegia genera and *Candida glabrata* species were overrepresented in CD whereas Leptosphaeria and Trichosporon genera were decreased. *Saccharomyces cerevisiae* and *Filobasidium uniguttulatum* species were associated with non-inflamed mucosa whereas Xylariales order was associated with inflamed mucosa. This study demonstrated the existence of an altered fungal microbiota in patients with CD.

Nystrom and colleagues [13] explored the mucosa-associated intestinal microbiome in children with treatment-naïve CD before and after receiving exclusive enteral nutrition (EEN). Mucosal biopsies from the ileum and the left colon were collected from eight children with CD who received EEN. All children were in clinical remission at the end of EEN, with endoscopic and histologic improvement but not full mucosal remission. In all children the mucosa-associated intestinal microbiome in both ileum and left colon changed radically with an increased overall bacterial diversity and a shift in microbiome composition, with decreased abundance in bacteroidales and enterobacteriales. The authors concluded that EEN could exert its effects by restoring mucosa-associated intestinal microbiome in pediatric CD.

CELIAC DISEASE

Marasco and colleagues [14] evaluated the fecal microbiota of 21 patients with celiac disease and compared it with 11 healthy controls; they also compared clinical parameters with bacterial levels in patients with celiac disease. The authors found a significantly greater abundance of Lactobacillaceae ($p<0.01$) and Streptococcaceae ($p<0.02$) cluster and a lower abundance of the Bacteroides-Prevotella cluster ($p<0.01$), Akkermansia ($p<0.01$), and Staphylococcaceae ($p<0.01$) in patients with celiac disease compared with healthy controls. Diarrhea was directly associated with Clostridium cluster IX ($p<0.01$), Bacillaceae ($p=0.03$), and of Fusobacterium ($p<0.05$). The presence of abdominal pain was associated with the abundance of Bacillaceae ($p<0.01$) and of Enterobacteriaceae ($p=0.01$). Furthermore, the abundance of Enterobacteriaceae was associated with anti-tissue transglutaminase IgA antibody levels ($p<0.05$). The authors concluded that the intestinal microbiota of celiac disease patients is different from that of healthy controls, with a particular abundance of potentially pathogenic species such as Enterobacteriaceae and Streptococcaceae, and also a depletion of Akkermansia, and a decrease of Bacteroides-Prevotella. Presence of GI symptoms was associated with a decrease of 'healthy' species and an increase in potentially harmful species.

CHRONIC PANCREATITIS

A study evaluated the gut microbiota in chronic pancreatitis (CP) and its association with Type 3c diabetes mellitus (T3cDM) and pancreatic exocrine insufficiency (PEI) [15]. Forty participants (16 with CP without T3cDM, 14 with CP and T3cDM and 10 healthy controls) were included and bacterial 16S rRNA was sequenced from the fecal samples. Amongst patients with CP, Bacteroidetes was higher in those with T3cDM compared with those without T3cDM. Faecalibacterium was found to be lower in patients with CP with T3cDM. In patients with CP, T3cDM and PEI, Bifidobacterium was significantly lower than those with T3cDM without PEI. They also found a positive correlation between Faecalibacterium, Ruminococcus, and glycemic status. Enterotyping was done for patients with CP with and without T3cDM. Enterotype 1 (Bacteroidetes predominant) was higher in patients with CP without T3cDM whereas Enterotype 2 (Prevotella predominant) was higher in CP with T3cDM. The authors conclude that PEI could be a contributing factor to dysbiosis in patients with both CP and T3cDM.

References

(1) Maharshak N, Tamar RK, Lundqvist A, Sartor BR, Carroll I, Ringel Y. Intestinal microbiota in patients with IBS-high throughput sequencing of the mucosa and luminal microbiota Presented at: United European Gastroenterology (UEG) Week; October 15-19, 2015; Barcelona, Spain.

(2) Lopetuso LR, Scaldaferri F, Petito V, Ponziani FR, Pecere S, Schiavoni E, et al. Gut microbiota molecular spectrum in healthy controls, diverticular disease, IBS and IBD patients: time for microbial marker of gastrointestinal disorders?. Presented at: United European Gastroenterology (UEG) Week; October 15-19, 2015; Barcelona, Spain.

(3) Tap T, Derrien M, Ohman L, Brazeilles R, Cools-Portier S, Dore J, et al. Identification of a gut microbial signature linked to severity of irritable bowel syndrome Presented at: United European Gastroenterology (UEG) Week; October 15-19, 2015; Barcelona, Spain.

(4) Cammarota G, Laniro G, Masucci L, Pecere S, Bibbo S, Scaldaferri F, et al. Fecal microbiota transplantation for recurrent C. difficile infection: a 2-year experience from a European referral centre. Presented at: United European Gastroenterology (UEG) Week; October 15-19, 2015; Barcelona, Spain.

(5) Scaldaferri F, Pecere S, Lopetuso LR, Laniro G, Laterza L, Schiavoni E, et al. An open-label, pilot study to assess feasibility and safety of fecal microbiota transplantation in patients with mild-moderate ulcerative colitis: preliminary results Presented at: United European Gastroenterology (UEG) Week; October 15-19, 2015; Barcelona, Spain.

(6) Hale V, Chen J, Rekdal V, Schmidt B, Khanna S, Jeraldo P, et al. A subset of patients with non-infectious diarrhea have altered gut microbiota similar to C. difficile infection. Presented at: United European Gastroenterology (UEG) Week; October 15-19, 2015; Barcelona, Spain.

(7) Kump PK, Wurm P, Grochenig H-P, Reiter L, Hoffmann KM, Spindelboeck W, et al. Impact of antibiotic treatment before faecal microbiota transplantation in chronic active ulcerative colitis Presented at: United European Gastroenterology (UEG) Week; October 15-19, 2015; Barcelona, Spain.

(8) Sandborn WJ, Gasink C, Gao LL, Blank MA, Johanns J, Guzzo C, et al. Ustekinumab induction and maintenance therapy in refractory Crohn's disease. N Engl J Med. 2012;367(16):1519-28.

(9) Scaldaferri F, Petito V, Lopetuso L, Pecere S, Paroni Sterbini F, Graziani C, et al. Anti-TNF-a induction regimen modulates gut microbiota molecular composition while inducing clinical response in Crohn's disease patients: toward a personalized medicine Presented at: United European Gastroenterology (UEG) Week; October 15-19, 2015; Barcelona, Spain.

(10) Magnusson MK, Strid H, Isaksson S, Lasson A, Bajor A, Ung KA, et al. The importance of the mucosal antimicrobial peptide expression and gut microbiota in anti-TNF therapy response in patients with ulcerative colitis Presented at: United European Gastroenterology (UEG) Week; October 15-19, 2015; Barcelona, Spain.

(11) Sokol H, Leducq V, Jegou S, Liguori G, Cosnes J, Seksik P, et al. Fungal microbiota dysbiosis in inflammatory bowel diseases patients Presented at: United European Gastroenterology (UEG) Week; October 15-19, 2015; Barcelona, Spain.

(12) Liguori G, Lamas B, Lavie-Richard M, Brandi G, Da-Costa G, Hoffmann TW, et al. Mucosal fungal microbiota dysbiosis in Crohn's disease Presented at: United European Gastroenterology (UEG) Week; October 15-19, 2015; Barcelona, Spain.

(13) Nystrom N, Wanders A, Frisk G, Finkel Y, Fuxe J, Bunikis I, et al. Exclusive enteral nutrition treatment restores the mucosa-associated intestinal microbiome in children with Crohn's disease Presented at: United European Gastroenterology (UEG) Week; October 15-19, 2015; Barcelona, Spain.

(14) Marasco G, Di Biase AR, Colecchia A, Schiumerini R, Biagi E, Brigidi P, et al. Gut microbiota in celiac disease patients and its correlation with symptoms Presented at: United European Gastroenterology (UEG) Week; October 15-19, 2015; Barcelona, Spain.

(15) Jandhyala SM, Vuyyuru H, Arutla M, Deepika G, Manohar M, Parimala V, et al. Study of the gut microbiome in chronic pancreatitis: association with pancreatogenic diabetes (type 3c) and exocrine insufficiency Presented at: United European Gastroenterology (UEG) Week; October 15-19, 2015; Barcelona, Spain.

20TH CONGRESS OF THE LATINO-AMERICAN SOCIETY FOR PEDIATRIC GASTROENTEROLOGY, HEPATOLOGY AND NUTRITION (LASPGHAN)

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INTRODUCTION

The 20th LASPGHAN Congress took place in Lima, the City of Kings, capital of Peru, and land of the Incas, from November 18th to 21st, 2015. There were 649 attendants, 213 of whom were Peruvian whilst the remaining attendants came from 27 different countries, mostly in Central and South America. The first day was structured as a pre-congress course aimed at general pediatricians. Topics of particular interest such as infantile colic, gastrointestinal urgencies, gastroesophageal reflux, and food allergies were presented by national and international experts. The main topics for this congress were hepatology, endoscopy, gastroenterology, and nutrition and consisted of a total of 75 sessions, including 5 plenaries. These were delivered simultaneously and most received good attendance.

For the first time, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) were invited to participate actively during the congress and each led a symposium comprising three sessions followed by three simultaneous additional sessions with members of LASPGHAN.

ESPGHAN SYMPOSIUM

The first session of the ESPGHAN symposium was presented by its president, Dr. Raanan Shamir (Israel), and was entitled "Can we prevent celiac disease?".
It examined factors to consider for the prevention of celiac disease and suggested a possible role for the intestinal microbiota, despite the current lack of evidence. Dr. Loreto Hierro (Spain) gave an interesting lecture on the "Transition of the hepatic patient and pediatric transplant to the adult system", describing her patients' experiences and sharing her expertise in this field of research. Finally, Dr. Jorge Amil (Portugal) gave a seminar on the current clinical state of celiac disease in pediatric patients in Europe.

The simultaneous sessions were:
1) "Introduction of solids (complementary feeding), recent evidence, new guidelines" by Ranaan Shamir (Israel) and Jorge Palacios (Guatemala),
2) "Chronic viral hepatitis: therapeutical strategies with new drugs" by Loreto Hierro (Spain) and Marcela Galoppo (Argentina) and,
3) "Eosinophilic esophagitis; current concepts", with the participation of Mario Vieira (Brasil) and Jorge Amil (Portugal).

NASPGHAN SYMPOSIUM

The NASPGHAN symposium was entitled "What is new in pediatric gastroenterology; news from north of the border". The current president of NASPGHAN, Dr. Carlo Di Lorenzo (USA), opened the symposium with the seminar "What is new in functional and motility gastrointestinal disorders". He provided some insight into the pediatric gastroenterology part of ROME IV classification, scheduled to be published shortly. Dr. James Heubi (USA) followed with "What is new in liver diseases in children" and highlighted interesting aspects of hepatology, drawing attention to metabolic diseases that may arise in the future. Finally, Dr. Francisco Sylvester, a Peruvian clinician who currently lives in the USA, presented a lecture entitled "What is new in inflammatory bowel disease in pediatrics", in which he highlighted the role of the microbiota in inflammatory bowel disease and new therapeutic strategies.

The aim of the simultaneous sessions was to expose divergent opinions from the North and the South of America with regard to the clinical approaches taken in the treatment of frequent pathologies in pediatric gastroenterology. "Constipation, what do we do in the North and what do we do in the South?" was presented by Dr. Di Lorenzo and Dr. Mauro Batista (Brazil), "Neonatal cholestasis, what do we do in the North and what do we do in the South?" was presented by Dr. Heubi and Dr. Mirta Ciocca (Argentina) and finally "Chronic diarrhea, what do we do in the North and what do we do in the South?" was presented by Drs. Sylvester and Fernando Sarmiento (Colombia), who emphasized the importance of considering the environment and lifestyle of patients with this pathology .

LASPGHAN WORKING GROUPS

For the first time, the LASPGHAN working groups communicated their findings. Two sessions, attended by many, gave the opportunity to members of the four working groups to share their results, which included: "Autoimmune hepatitis, fulminant hepatitis" by Mirta Ciocca (Argentina), "Eosinophilic esophagitis", by Reinaldo Pierre (Venezuela), "Inflammatory bowel disease", by Mónica González (Chile); and "Infantile metabolic syndrome", by Fernando Sarmiento (Colombia).

ABSTRACTS

A total of 215 abstracts were submitted and, according to tradition, ten of the best were chosen to be presented orally in two forums, in honor of Horacio Tocalino and Victor Martin Campos who founded the Society in 1975. From these, two were selected for awards; in the Tocalino Forum, Lucero Alvarez, from Chile, won the award for his contribution to "Infection by *Helicobacter pylori* cagA+ and severity of damage in duodenal histology in patients with celiac disease". In the Martin Campos Forum, Rosa Lama, from Spain, won the award for her contribution to "Multicenter clinical trial in children with non-organic failure to thrive: nutritional and inflammatory response with a hypercaloric formula with symbiotic and DHA&ARA".

CONCLUSION

Despite the unusually low temperatures in Lima for the time of year, the exciting scientific line-up, the cultural diversity of attendants, the engaging social activities and, last but not least, the world-famous Peruvian gastronomy all contributed to a warm, welcoming, and stimulating LASPGHAN Congress 2015. We look forward to the next LASPGHAN Congress, which will take place in Porto, Portugal, in 2017.

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