Gut microbiota composition: A new kind of biomarker?

By Andreu Prados

At the last Gut Microbiota for Health World Summit 2016 that was held in Miami, USA March 5-6, 2016, leading experts presented up-to-date evidence on clinical applications of gut microbiota and its mechanisms and role in human health and disease. **The composition of the gut microbiota is emerging as a new indicator or marker of health**, as it could in the future be used as a sensitive tool for identifying nutritional habits as well as disease risks. This is based on current evidence that composition and functions of the human gut microbiota are significantly altered with different nutrient intake, and also in patients with chronic gastrointestinal (GI) disorders, liver disease, and several metabolic and autoimmune conditions. Thus, microbial communities inhabiting the human gut provide a huge potential for new biomarkers. This report aims to show recent research findings in the field of gut microbial biomarkers: how gut microbiota can provide insights into the onset as well as in the management and treatment of conditions.

Colon cancer risk

Dietary factors and the associated lifestyle are considered the major regulators of the gut microbiota. Thus, gut microbiota composition might be used as a sensitive tool for identifying nutritional habits as well as disease risks that are related to these habits. During the congress, Kishore Vipperla from the Division of General Internal Medicine at the University of Pittsburgh (USA), showed that **a change in diet composition produces immediate effects on the metabolic phenotype of the colonic bacterial contents, which are associated with reciprocal mucosal biomarkers of cancer risk**. His research showed **gut microbiota can be rapidly altered by changes in diet.** According to the study that was presented, led by Stephen J.D. O'Keefe's team from the Division of Gastroenterology, Hepatology and Nutrition at University of Pittsburgh (USA), **westernization has a significant impact on mucosal biomarkers of cancer risk, which appear to be regulated by gut microbiota**. This study enrolled a group of 20 African American volunteers and another group of 20 rural South Africans; the latter group is known to have low rates of colon cancer (<5:100,000). Americans typically had a diet high in animal protein and fat, whereas Africans had a diet high in fibre–mainly in the form of resistant starch–and low in fat and protein. When subjects' diets were swapped for just two weeks, researchers showed that reciprocal changes occurred in mucosal biomarkers of cancer risk and microbial metabolites involved in diet-associated cancer risk. It was noteworthy that adopting a typical African diet increased saccharolytic fermentation and butyrogenesis and suppressed secondary bile acid synthesis, the latter being associated with cancer risk both in experimental models and in human studies. According to this research, **gut microbiota composition might be used as a biomarker for nutritional habits as well as for assessing possible cancer risk.**

Christian Jobin from the Division of Gastroenterology at University of Florida College of Medicine (Gainesville/USA) focused on the impact of certain *Escherichia coli* strains in the onset of colitis and colorectal cancer (CRC). Jobin has studied the differential contribution of bacteria to protecting against or exacerbating colitis and CRC; as he pointed out, **adherent-invasive *E. coli* (AIEC) strain, which shows a higher prevalence in inflammatory bowel disease (IBD) and CRC patients compared to healthy subjects, has the ability to adhere to the epithelial cells and subsequently invade the intestinal mucosa**. Beyond inducing inflammation environment and using by-products of inflammation as energy sources, AIEC produces a genotoxin called colibactin that promotes inflammation and carcinogenesis; this has been confirmed in preclinical models. Colonization of the gut with AIEC is enhanced by a western type diet and, therefore, nutritional habits may impact health through gut microbiota modulation. As the risk for developing CRC is higher in patients with inflammatory bowel diseases, Prof. Jobin concluded that investigating host-microbiota relationships in IBD and CRC it will allow us to develop novel strategies to detect and treat these conditions.

Inflammatory bowel disease

R. Balfour Sartor, from the University of North Carolina School of Medicine (USA), discussed the role of gut microbiota and genetics in IBD. **Genetic impacts on the development of IBD were said to be important but, alone, genes cannot cause the disease**. This is based on the fact that monozygotic or identical twins in Crohn's disease have only a 50% concordance, meaning that if one twin has Crohn's disease the other twin has Crohn's Disease only in 50% of the cases, whereas in ulcerative colitis the concordance is only 10%. This shows environmental factors are necessary to trigger the disease. Dr. Sartor pointed out that **western diet and processed foods (high in fat and low in fibre) along with a wide use of antibiotics could help explain the rapid increase in prevalence and incidence of IBD globally,** and especially in developing countries in Asia, South America, and Africa. Besides this, Dr. Sartor emphasized that the impact of gut microbiota on the development of IBD is more certain in animal models; yet not all bacteria stimulate aggressive immune responses with a subsequent tissue injury: some microbiota compositions are protective. There may be identifiable signatures in the gut microbiota that could be useful for indicating IBD. **Determining an artificial group of bacteria that can be transplanted would help targeting IBD, although currently there is no conclusive data for using faecal microbiota transplantation in Crohn's disease and ulcerative colitis.**

Liver disease

Bernd Schnabl, from the Department of Medicine at the University of California, San Diego (USA), showed **the gut microbiome could have diagnostic significance in liver disease**. Schnabl focused on the link between alcohol, liver disease, and the gut microbiome, and explained that the role of gut microbiota in liver diseases was supported by the fact that

transferring the microbiome of patients with alcoholic hepatitis into germ-free mice induces liver injury in mice. What is more, he explained, intestinal microbiota contributes to individual susceptibility to alcoholic liver disease. Schnabl said that a permeable gut barrier in patients with liver diseases leads to translocation of bacterial products that promote hepatic injury and inflammation.

In addition, chronic alcohol consumption can change gut microbiota composition by causing small intestinal bacterial overgrowth (SIBO); **alcohol-associated dysbiosis could be partially reversed after a few weeks of alcohol abstinence both in mice and in humans**, although this phenomenon has not been followed long-term yet. In this context, he mentioned that dietary patterns have a very profound effect, as mice studies showed that supplementing diet with saturated fatty acids may prevent alcoholic liver disease.

When it comes to treating liver disease, Schnabl **highlighted that stabilizing the gut barrier is highly effective in addressing symptoms in liver diseases, and that probiotics could have a promising role by helping restore the gut microbial community**. On the other hand, **non-absorbable antibiotics that decrease the amount of intestinal bacteria may manage alcohol-induced liver disease and its associated dysbiosis, though precision microbiome therapies are needed**. Identification of targets in the gut microbiome, which could be bacteria themselves, genomic function, or their metabolites, will be important for limiting the progression of liver disease. For example, Schnabl's research group has demonstrated that intestinal levels of the antimicrobial-regenerating islet-derived (REG)-3 lectins protect against alcoholic steatohepatitis by reducing mucosa-associated microbiota and preventing bacterial translocation. Thus, **strategies to restore levels of intestinal REG3 lectins might be developed to treat alcoholic liver disease**.

On the whole, **microbiota composition and metabolites could one day be used as biomarkers of liver diseases, and could be manipulated exogenously for achieving clinical improvement**.

Pregnancy complications

Recent work has suggested the possible role of gut and vaginal microbial communities as biomarkers of health problems during pregnancy. But before scientists can identify microbial markers of pregnancy complications, they must understand how the gut microbiota changes throughout the course of a healthy pregnancy. During the Gut Microbiota for Health World Summit 2016, Omry Koren of the Faculty of Medicine, Bar-Ilan University, Israel, presented data on what happens to gut microbiota during pregnancy. It was shown that pregnancy progression is associated with hormonal, metabolic, and immunological changes that occur in parallel with dramatic alterations in the composition of the gut and vaginal microbiotas. The vaginal microbiota of pregnant women is characterized by a decrease in bacterial diversity (alpha diversity), which is also seen in the gut microbiota as pregnancy progresses.

Koren showed that in the gut, the lower diversity is accompanied by an increase in "between sample" diversity (beta diversity) and an increase in the number of Proteobacteria and opportunistic pathogens. **Some metabolic changes during normal pregnancy are similar to aspects of metabolic syndrome**. Germ-free mice inoculated with gut microbiota from pregnant women presented with metabolic changes mirroring those of the pregnant women (e.g., greater adiposity and insulin resistance in the third trimester). Koren pointed out that it is not currently clear whether these changes in microbial communities are a cause or consequence of some of the characteristics of third-trimester pregnancy. Besides this, the contribution of these bacteria to complicated and uncomplicated pregnancy deserves further research. New questions are emerging, such as whether the changes in gut microbiota composition are gradual or sudden, and what is the interplay between gut microbiota and the endocrine and immune systems during pregnancy. On the whole, **gut microbiota composition could mirror physiological phenomena that take place during pregnancy and, therefore, its study could provide us with opportunities to intervene with bacteria-tailored therapies (e.g. probiotics) for better health of mother and child.**

Programming of health in early life

Maternal health status, mode of delivery, and feeding practices are emerging as major drivers of early programming of an infant's immune and metabolic phenotype. If microbial markers of later disease risk can be identified, early life may represent a window of opportunity to modify the programming process to reduce the risk of disease in later life. Jose Carlos Clemente, from the Department of Medicine of the Icahn School of Medicine at Mount Sinai in New York (USA), highlighted that an infant's **delivery mode is a major determinant of the microbiota composition**. For many reasons, the incidence of caesarean sections (c-sections) is increasing across the world. C-sections raise the risk for asthma, as well as metabolic and immune deficiencies in adulthood; Clemente and his group are exploring whether the gut microbiota contributes to this risk, and whether it could be modified early in life for better health outcomes. A recent paper of Clemente and colleagues showed a technique that allows a vaginal-birth-like community of **microbes to be partially restored at birth in babies delivered by c-section**. As all mothers in the study breastfed, diet was not a confounding factor in the study. Clemente concluded that partial restoration of vaginal microbes could be used for a therapeutic purpose; however, he emphasized that long-term studies are required to understand their impact on health.

Victoria Ruiz, from the team of Martin Blaser from the Department of Medicine at New York University School of Medicine, presented the consequences of early-life antibiotic exposure on intestinal microbiota and host immunity. One noticeable fact she presented was that **antibiotic use in early life, even once, can change the dynamics of the gut microbiota and has long-term effects in mice**. Her research studies have focused on metabolic and immunologic consequences of antibiotic exposures pre-, peri-, and post-partum, and provide evidence that early-life therapeutic doses of

antibiotics or pulsed antibiotic treatments affect the composition of the microbiome, and have phenotypic consequences for the offspring. The phenotypic changes may persist after microbial recovery. Identifying key markers of disturbance and recovery in infants may help in providing therapeutic targets for microbiota restoration and mitigation of health risks following antibiotic treatment.

Metabolic disease

Regarding the role of gut microbiota in development of obesity and insulin resistance, Max Nieuwdorp from the Academic Medical Centre in Amsterdam, The Netherlands pointed out that **specific changes of the microbial composition in patients with metabolic disease might be potentially used as biomarkers of their condition**. This is based on the fact that there is a link between metabolic disease development and intestinal microbial composition. However, the impact of anti-diabetic medications on the gut microbiota may be a confounding factor, since it has been demonstrated that subjects with type 2 diabetes mellitus (T2D) who used the anti-diabetic drug metformin had increased levels of *Enterobacteriaceae* and decreased levels of *Clostridium* and *Eubacterium* compared to those without this medication. Nieuwdorp presented several studies in which several bacteria, such as *Lactobacillus gasseri* and *Streptococcus mutans*, serve as a good predictor for the development of insulin resistance, a well-known precursor of T2D. Besides this, a unified signature of gut microbiome shifts in T2D was reported, with a depletion of butyrate-producing taxa. Thus, the study of changes in microbial composition in metabolic disorders, including T2D and obesity, will help to advance toward microbial biomarkers for early diagnosis of the disease. Although the diagnosis of the condition was traditionally based in classical biomarkers such as fasting blood sugar and HbA1c, **recent studies suggest that examinations of microbial changes may potentially be used as a complementary biomarker for identifying individuals who are at risk of developing metabolic diseases**.

Faecal transplantation studies in conjunction with faecal sampling in prospectively followed cohorts will help identify causally involved intestinal bacterial strains in obesity-related disorders. Niewdorp's research experience with faecal microbiota transplantation (FMT) has shown that it can be used for improving insulin sensitivity (but not weight) in patients with metabolic syndrome. Overall, **data presented by Nieuwdorp emphasizes that in the future gut microbiota interventions might be novel therapeutic strategies in the treatment of obesity and obesity-related disorders**. Therapies for the future might include reconstitution of missing bacterial strain(s), dietary intervention, or administering metabolites.

Brain disorders

Beyond using gut microbiota composition for identifying GI system and metabolic disorders, **recent advances suggest a possible role for the gut microbiota as a biomarker of brain-related conditions.** The human gastrointestinal tract contains a large and complex neural network called the enteric nervous system, whose main purpose is to regulate the physiological functions of the gut (motility, secretion, absorption). Communication between the brain, the GI tract (including gut microbiota), the endocrine system, and the immune system is governed by the so-called gut-brain axis.

Gut microbes appear to influence brain development and behaviour, and the brain-gut-microbiota axis plays an important role in regulating stress responses. Timothy G. Dinan, from the Psychiatric Department at University College in Cork (Ireland), **emphasized the potential of psychobiotics in managing stress-related conditions and improving cognition**. He is the first person who has defined the concept of a psychobiotic as a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness. A subset of probiotics, these bacteria could be capable of producing and delivering neuroactive substances such as gamma-aminobutyirc acid (GABA) and serotonin, which act on the brain-gut axis. **Altered communications between the gut microbiota and the brain may play an important role in human brain disorders** such as autism spectrum disorders (ASD) as well as depression and anxiety. Although mechanisms of psychobiotics may be plausible, Dinan emphasized that large placebo-controlled studies are needed in order to study the possible beneficial effects of probiotics in disease states involving the brain or the gut/brain axis.

Regarding the latest advances about gut microbiota and ASD, Dr. Emeran Mayer, from the Oppenheimer Centre for Neurobiology of Stress and Resilience at University of California, Los Angeles (USA), covered the increased GI symptoms in ASD and the alterations in gut microbial composition and related metabolites in some ASD patients, which may be influenced by diet, central factors, or early life programming of the gut microbiota. The maternal immune activation (MIA) mouse model of ASD has good face and construct validity for ASD. Besides this, **findings of altered serum metabolite profiles in the MIA mouse model raise the possibility of testing particular metabolites as candidate biomarkers for subsets of human ASD**. However, it remains to be determined if gut microbial influences play an important role in human brain development and to what degree they can influence the function of the adult brain and associated behaviours.

Concluding remarks

To sum up, **scientific evidence supports microbiota composition as an emerging potential biomarker or indicator of health. It might help predict risk of chronic diseases such as metabolic syndrome; it might aid in early identification of colon cancer; also, it may mirror intestinal inflammation both in normal and pathological states.**

Furthermore, microbiota interventions might offer a new opportunity for targeting chronic diseases in the future. Increasing our knowledge about the interactions between commensal microbiota, host immunity, and host metabolism–with environmental factors influencing each component of this triad–may result in a better understanding of diseases, even beyond the gastrointestinal tract, and finally lead to better preventive and therapeutic strategies.

Current research approaches could provide a basis for the definition of a 'healthy' gut based on key properties of microbial functionality. This will enable the development of direct nutritional strategies for intestinal disease prevention and health promotion. In the next few years, the results of international projects designed to determine the precise impact of the microbiota in various pathological processes will hopefully lead to major advances. **The challenge will be to gain a better understanding of the mechanisms linking microbiota composition and activity with physiological and pathological processes and how these interactions may be modified by diet to support the health of the human host.**

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