Biome profiling and clinical parasitology: tools for gut microbiota restoring

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Abstract: A growing body of evidences is showing that dysbiotic gut microbiota may correlate with a wide range of disorders; hence, the clinical use of microbiota maps and fecal microbiota transplantation (FMT) can be exploited in the clinic of some infectious diseases. Through direct or indirect ecological and functional competition, FMT may stimulate decolonization of pathogens or opportunistic pathogens, modulating immune response and colonic inflammation, and restoring intestinal homeostasis, with reduction of host damage. Herein, we discuss how diagnostic parasitology may contribute to design clinical metagenomics pipelines and FMT programs, especially in pediatric subjects. The consequences of a more specialized diagnostics in the context of gut microbiota communities may improve the clinical parasitology and extend its applications to the prevention and treatment of several communicable and even noncommunicable disorders.

Keywords: parasites; microbiota; faecal microbiota transplantation (FMT)

Introduction

The intestinal environment is an ecosystem where biological and chemical interactions occur at various organizational levels between host, parasites, and microbial communities, greatly affecting human health and physiology.

Considering the gut microbiota at taxonomic level, we can observe a significant variation among individuals, each harboring a unique collection of bacterial species, which may change over time and could be considered such a fingerprint [1–3].

The microbiota at level of the gut microenvironment provides important protective, immune regulatory and metabolic functions. The defensive mechanism against pathogenic bacteria is exerted by the barrier effect of the intestinal epithelium, playing a major role in protecting the host, and really representing an important obstacle to pathogens invasion [4,5].

Moreover, environmental factors, such as age, diet, stress, drugs, strongly influence the composition of the human microbiota [6–9].

The balance of the gut microbial ecosystem, eubiosis, is an important concept. An eubiotic gut microbiota is characterized by a preponderance of potentially beneficial species, belonging mainly to the two bacterial phylum Firmicutes and Bacteroides, while potentially pathogenic species, such as Proteobacteria. In the dysbiosis state, “bad bacteria” predominate “good bacteria” [10–12].
Within this complex scenario, intestinal citizens (e.g., virus, mycetes and parasites) interact with the microbial community modifying the balance between host and gut microbiota [4,13].

The intestinal microenvironment considered as a whole community provides an important protective, mucosal defense mechanism, but there are evidence that change in the composition of the commensal microbiota alters the gut environment making this composition vulnerable to pathogenic organisms [14–16]. Many factors such as antibiotics, psychological stress, physical stress, modern diet and hygiene can affect the microbial stability, and thus, contribute to the intestinal dysbiosis [11]. Indeed, dysbiosis may be harmful for the host, leading to inflammation and mucosal tissue damage that predispose to pathological conditions as in the case of Clostridium difficile infections [17].

2. Diseases and gut related microbiota profiling

C. difficile is an infectious Gram-positive spore forming bacillus microorganism of the gastrointestinal tract, and its toxin expression causes gastrointestinal illness with a wide spectrum of severity, ranging from mild diarrhea to pseudomembranous colitis, toxic megacolon, sepsis and death [18,19]. C. difficile is considered a member of the normal gut microbiota, however its growth is suppressed by other more dominant anaerobes [17]. The gut colonization of C. difficile is reversely related with host age, growing in early infancy and decreasing in adulthood, and it depends on the loss of the commensal microbiota barrier effect following, for example, antimicrobial therapies. In this context, C. difficile infection is responsible for initiation of the cascade of inflammatory processes which may play an important and destructive role in initiation and perpetuation of intestinal inflammation [17].

Patients with recurrent C. difficile infections are characterized by almost monomicrobial bacterial distributions of the fecal microbiota [20]. A decrease in bacterial diversity and a strong variation in global distribution of operational taxonomic units (OTUs) are, indeed, registered in the fecal microbiota profiling of adult patients presenting with C. difficile infections [20]. In particular, a statistically significant increase of Firmicutes and a decrease of Bacteroidetes phyla, compared to healthy subjects or controls (CTRLs) are observed, as well as an increase in Clostridiaceae and Erysipelotrichaceae families (Figure 1). Remarkably, in microbiota profiles associated to infectious agents, the dysbiosis index (DI) is usually very high, based on the prevalence of few microbial taxa, as in the case of the maps reported for C. difficile infection, for which observed dysbiosis values actually range in very high value intervals (DI>35%) (Lorenza Putignani and Antonio Gasbarrini, oral communication, 24°Congresso Nazionale delle Malattie Digestive, Rome, FISMAD March 23, 2018) (Metagenomic Method for the in vitro diagnosis of intestinal dysbiosis, PTC/IT2017/000119 Patent).
Figure 1. Faecal microbiota map of a patient affected by a Clostridium difficile infection. Panel A and B describe global distribution of microbiota map at Phylum and Family taxonomic levels. Red and green arrows, refer to decreased and increased, respectively, microbial signatures associated to the disease-driven profiling.

Another powerful microbial marker associated to different diseases is Akkermansia muciniphila. A. muciniphila is not a specific disease-related marker, but can be found across disease profiling and depending on host age; it is generally depleted in faecal samples from subjects suffering from a variety of diseases, including obesity, metabolic syndrome, diabetes, cancer, generally characterized by high levels of inflammation [21,22].

The reduction of A. muciniphila following antibiotic treatment has been associated to a reduction in the expression of Muc2 gene encoding the major mucin of the colonic mucus in colonic tissues, as well as reduced mucolysis [23]. Indeed, the analysis of mucosae showed A. muciniphila to be abundant in biopsies of healthy subjects but reduced in those of patients with inflammatory bowel disease (IBD), who in contrast, had increased amounts of Ruminococcus [24].
The turnover of mucus (synthesis and shedding) [25] plays a crucial role by spatially protecting the intestinal cells of the intestinal barrier by bacteria flow coming from the luminal content, activity reinforced in some cases by the production of secretory immunoglobulin A (sIgA), and secretion of antimicrobial peptides and proteins [26]. The depletion of *A. muciniphila* is observed, as reported above, in several diseases and our group has thoroughly worked on its role in Nonalcoholic Fatty Liver Disease (NAFLD) and liver disease progression, such as non-alcoholic steatohepatitis (NASH) [11,27,28] (Figure 2) and in lung cancer (Figure 3). [29,30]

Figure 2. Faecal microbiota map of a patient affected by non-alcoholic steatohepatitis (NASH) and severe insulin resistance. Panel A and B describe global distribution of microbiota map at Phylum and Family taxonomic levels. Red and green arrows, refer to decreased and increased, respectively, microbial signatures associated to the disease-driven profiling. The map is associated to a 27% DI.
Figure 3. Faecal microbiota map of a patient affected by non-small cell lung cancer undergoing therapy with Nivolumab. Panel A and B describe global distribution of microbiota map at Phylum and Family taxonomic levels. Red and green arrows, refer to decreased and increased, respectively, microbial signatures associated to the disease-driven profiling.

Indeed, the idea to modulate *A. muciniphila* as remodeling intervention for gut dysbiosis or as tool to potentiate chemotherapy or immunotherapy [31] are under the magnifying class of the current advanced research [32]. However, the interplay between bacterial biomarkers, their metabolic and immunological functions and other components of the gut microbiota is the next challenging aim of the translational and clinical studies on microbial communities.

3. Role and Relationship amongst gut microbiota citizens

In addition to bacteria, other key microorganisms, such as yeasts and filamentous fungi, viruses, phages, are present in the gut [33,34]; moreover, for ~25% of the world’s population, the gut microbiota also comprises intestinal protozoan and worms, namely meiofauna [35,36].
The diversity of meiofauna living on or in our bodies is associated to all metazoans with dimensions between 30 µm and 1 mm. Many members of the meiofauna significantly affect morbidity and mortality, including fungi (e.g., Candida, Aspergillus), unicellular protozoa (e.g., Giardia, Entamoeba), and helminthic worms (e.g., Ascaris) (Figure 4) [37].
Figure 4. Simplified taxonomic overview of the meiofauna of the human GI tract.
Sequencing technologies are allowing to analyze the global diversity of the meiofauna of the human gastrointestinal tract to intimately demonstrating that commensal gastrointestinal meiofauna may be important in promoting health or disease. Several studies indicate that diet can influence the proportions of the meiofauna and that there is the possibility of trans-kingdom interactions in the gastrointestinal tract [37].

All these microorganisms offer additional dimensions to the investigation of host-microorganisms’ and microorganisms-microorganisms’ interactions; interplays that can be exploited only by combined models obtained by metagenomics, metabolomics and metaproteomics armonization and integration [38], aiming at producing decision support systems for disease stratification in medicine [39].

All microbial inhabitants may have both beneficial and detrimental roles in the human health, including improvement of microbial resilience, immune evasion, maintenance of physiologic processes, but even alteration of microbial communities [40]. Bacteriophages in the gut are largely unexplored, despite their potential to regulate bacterial communities and thus human health [41,42]; indeed experimental limitations still affect successfully isolation of phages and genome annotation as well as the full characterization of virus human populations [43]. Some studies have highlighted the uniqueness of phage communities in individuals and their capacity to be stable in the healthy gut [44]. Remarkably, the stability of the viral genome is probably responsible for the stableness of bacteria and microbiota metagenomes [45]. However, still there are only few studies carried out on the role of the virome in the host’s intestinal microbiota ecosystem [46].

Fungi are normal inhabitants of the mammalian gastrointestinal tract. In fact, the human gut is colonized by more than 50 genera of fungi [47]. In particular, the gut is characterized by the presence of Candida, Saccharomyces and Cladosporium species. Nutritional modification may have an effect on the fungal microbiota; in particular, plant-based diets increase level of Candida, whereas animal-based diets improve the presence of Penicillium species [48,49]. Indeed, fungi compose a very small portion of gut microbiota, but play determinative roles in homeostasis of the gut bacterial composition and the mucosal immune responses. An interkingdom correlation between bacteria and fungi has been suggested. Alterations in the composition and function of the gut microbiota are a usual event in patients who suffer from IBD. Although the main reason for this alteration is not clear, the interaction between gut bacteria and gut fungi seems to be an important subject in IBD patients [50].

Intestinal parasites, both protozoans and metazoan (helminths and cestoda), interact with the microbial community modifying the balance between host and gut microbiota [51] (Figure 5).
Figure 5. Human gut biome characterized by bacteriome, virome and meiofauna.

The microbiota of host may strongly interfere with the survival and the physiology of many parasites and, consequently, with the outcome of many parasitic infections. A wide range of protozoans are common parasites of the human gastro-intestinal tract (e.g., *Cryptosporidium* spp., *Entamoeba histolytica*, *Giardia duodenalis*). The spectrum of clinical manifestations of protozoan infections varies from a chronic diarrhea or weight loss or mild self-limiting illness to acute disease, until malabsorption [36,52,53]. Since the composition of the intestinal bacterial population affects the impact of the infection of protozoans, the modulation of different components of the microbiota could be used to prevent or attenuate intestinal protozoan infection and ultimate outcome of parasitic disease.

4. Parasites and gut microbiota profiling

Parasitic infections represent a significant health problem, particularly in underdeveloped and developing countries. Soil-transmitted helminths (STHs), e.g. *Strongyloides stercoralis* and *Trichuris trichiura*, are usual intestinal parasites, followed by blood flukes (*Schistosoma* spp.) and filarial worms (e.g., *Wuchereria* and *Brugia*). Generally spoken, intestinal parasitic infections have a low-impact in healthy subject; however, they can become a potential major issue in vulnerable groups. In fact, helminths infection produce malnutrition, physical damage and cognitive development in children [54].

In fact, together with trillions of microorganisms, *i.e.*, archaea, viruses, bacteria, and eukaryotes residing in the GI tract, parasitic worms establish the “macrobiota” [55]. During parasitic infections, the interaction between the gut microbiota and the helminths, has been associated with the establishment of the infection, the clinical manifestations and even the immune-modulation [56,57].
Experiments led in murine models of intestinal schistosomiasis have suggested the interactions between *Schistosoma* parasites and the host gut microbiota with a direct impact on the intestinal microbial communities [58]. In a study of Alba Cortés et al. the gut microbiome composition of the host actually influenced the host susceptibility to *S. mansoni* infection, as well as infection-associated changes in gut microbiota profiles [58]. Some studies have evidenced that *Necator americanus* infection could alleviate chronic inflammation in celiac disease and improve prokaryotic species richness, reestablishing the eubiosis and immune homeostasis [59]. In a case report on *Strongyloides stercoralis* infection, gut microbiota composition triggered an enrichment in *Bifidobacterium, Blautia, Ruminococcus, Bacteroides, Corynebacterium, Clostridium, Streptococcus, Coprococcus,* and *Oscillospora* genera, and a decrease in *Staphylococcus, Lactobacillus,* and *Pediococcus.* The authors suggested a putative direct or immune-mediated ability of *S. stercoralis* to promote the increase in bacterial diversity [60].

A review of the literature tried to deepen the mutual influences of intestinal nematodes and host-gut microbiota, highlighting the potential benefits effects (i.e., promotion of eubiosis) through the production of useful metabolites (i.e., short-chain fatty acids, SCFA). In other way, nematodes’ infection promote dysbiosis due to promotion of pathogenic bacterial species and decrease of mutualistic commensal. Authors exemplified the nematode-microbiota interactions and their impact on the host immune response [61].

A shotgun metagenomics study on samples of patients with the *Blastocystis* spp. infections, founded a very strong association between the presence of *Blastocystis* spp. and the abundance of archaeal organisms (*Methanobrevibacter smithii*) [62]. Additionally, another study showed a decrease of *Blastocystis* in individuals with the *Bacteroides* enterotype compared to subjects with the *Ruminococcus* or *Prevotella* enterotypes [63].

In a rat model of *Blastocystis* ST3 infection, the colonization altered gut microbiota composition, but not richness, inducing only mild gut inflammation but no clinical symptoms. In addition, the long-term *Blastocystis* exposure appeared to promote a faster recovery from colitis, suggesting that *Blastocystis* may alter the gut ecosystem in a protective way and promote faster recovery [64]. On the opposite, in healthy subjects, the fermentation by anaerobic bacteria and *Blastocystis* induced an increased SCFA production [65].

In the January 2019 started the Parasite Microbiome Project (PMP) to understand the role of parasite-associated gut microbiota on the pathophysiology of helminthiasis [66]. The PMP tried to draw best practices for experimental studies to ensure reliable comparisons between data sets and the introduction of appropriate controls to identify possible environmental microbial contaminants [66]. In a recent review, the authors suggested four elements, which must be considered when the scientist wants to generate reliable and reproducible data [55]. In a first point, they propose to generate an appropriate negative controls (“blanks”) in each step of the experiment, followed by the microscopy-based visualization of helminth-associated bacteria, to identify and characterize worm microbiomes across different helminth tissues and developmental stages. Remarkably is the identification of the “core microbiome” in helminth tissues versus transiently associated bacteria, in a way to depict the specific function of the only helminth-associated microbiome. These best practices are becoming more and more important, because clinical metagenomic next-generation sequencing (mNGS) is rapidly moving from research to clinical laboratories. Indeed, Chiu et al., focused on the challenges of implementing mNGS in the clinical laboratory and to address potential solutions for maximizing its impact on patient care and public health. Clinical applications of metagenomic sequencing include direct identification of microorganisms from primary clinical samples, antimicrobial resistance prediction by characterization of resistance genes, detection of species-level or strain-level virulence determinants, and antiviral resistance prediction. Furthermore, by means of NGS techniques it is now possible to analyze the onset and progression of infectious diseases in acute and chronic stages [67]. The current challenge will became the agnostic approach trough the complete characterization of enteric microbial communities, shallow complete metagenomics and trans-kingdom complete metagenomics (Figure 6).
Figure 6. An example of gut parasitome kronos graphs obtained by applying agnostic metagenomics pipelines. Panel A: parasitome superkingdom; Panel B: parasitome, no rank; Panel C: parasitome phylum.

5. Parasites and fecal microbiota transplantation (FMT)
FMT has a major role to manage important diseases such as *C. difficile* infections. In these cases, the transplantation is followed by a re-establishment of diversity; and, in many cases, the percentage of efficacy has been greater than 90% [68]. FMT is considered as the “ultimate probiotics” because it directly changes the intestinal microbial composition of the host, thus restoring eubiosis and intestinal homeostasis. FMT donor screening is a key factor in the safety of the procedure in order to prevent iatrogenic infectious diseases potentially transmittable to the recipient [69]. In fact, the international consensus on stool banking for FMT established that donor stool must be tested for protozoa and helminths, including *Blastocystis hominis*, *Dientamoeba fragilis*, *G. duodenalis*, *Cryptosporidium* spp., *Isospora* and *Microsporidia* [70]. In addition, the donor must be negative for blood nematodes test (*i.e.* S. stercoralis) [70] and a particular attention is necessary for parasitological screening of donors when recipients are children [71]. Remarkably, the fecal material (*i.e.*, emulsion) can be stored frozen in a stool bank for use when needed [71].

Indeed, FMT requires a combination of expertise and appropriate methods, including clinical parasitology, to identify the best donors.

### 6. Materials and Methods

A literature review was conducted to analyze the role and effects induced by microorganisms (*e.g.*, bacteria, viruses, fungi, worms and protozoa) on the microbial communities of the gastrointestinal tract both in conditions of eubiosis and dysbiosis. The research was conducted on PubMed and using the following terms: “virus” or “fungi” or “worms” or “protozoa” or “diet” or “nutritional status” or “microbiota” or “microbiome” or “dysbiosis” or “eubiosis” and “gastrointestinal symptoms”. All articles providing sufficient information about the relationship between the gut microbiota, NGS, clinical parasitology, FMT. The inclusion criteria for study were as follows: (1) observational prospective and retrospective studies, case–control studies, cohort studies or systemic review; (2) study investigating gut microbiota profiles and parasites infection; (3) studies written in English. All the studies that did not fall in the following criteria were excluded from the reviewing process.

### 7. Conclusions

The consequences of a more specialized diagnostics in the context of the characterization of gut microbiota communities may improve the clinical parasitology and extend its applications to the prevention and treatment of several communicable and even non communicable disorders.

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